SEASONAL CHANGES AND RELATIONSHIPS BETWEEN AEROBIC CAPACITY, HEART RATE VARIABILITY AND IRON STATUS IN JUNIOR CROSS-COUNTRY SKIERS

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ABSTRACT

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Over the years, athlete monitoring has become a standard practice in helping athletes reach their peak performance. The several purposes for athlete monitoring include determining training adaptations and finding the balance between training and recovery. In endurance sports, where both training volume and intensity are relatively high, the monitoring of training is especially important to help maximize performance while ensuring sufficient rest and recovery. Maximal oxygen uptake (VO_{2max}) is a widely used variable in estimating aerobic capacity while monitoring of recovery is a more complex process. There are several ways to define recovery status, including detecting changes in heart rate variability (HRV) and body iron status, which are very different but both commonly used measures among endurance athletes. The purpose of this study was to examine how aerobic fitness and recovery vary in junior female cross-country skiers before and after six-months long training season and to detect the relationships between aerobic capacity, HRV and iron status.

Methods. Ten junior female cross-country skiers participated in the study. The study protocol included two testing-periods that occurred nearly six months apart from each other at the beginning and at the end of the athletes' training-season. The two testing-periods lasted from one-to-two weeks and included an incremental maximal aerobic fitness test, nocturnal HRV recordings and iron status measurements. Incremental maximal aerobic fitness test was used to quantify aerobic capacity (VO_{2max}). The nocturnal HRV was measured as a weekly average with a contact-free sleep tracking device that reported the magnitude of HRV with a time-domain variable RMSSD. Iron status was evaluated by using Hb_{conc}, HCT and s-Ferr that were obtained from blood samples drawn from the antecubital vein.

Results. There were no significant changes in any of the measured variables between the PRE and POST measurements. Relationships between recovery markers were, however, prominent since there were significant positive correlations between changes in HRV and functional iron Hb_{conc} (0.796, $p<0.01$) and between HRV and HCT (0.717, $p<0.05$). Although there were no associations between VO_{2max} and the recovery markers in the whole study group, individual cases reveal how two subjects, whose Hb_{conc} decreased, had either impaired or unchanged VO_{2max}. The two subjects with decreased Hb_{conc} values had impaired results in all the recovery markers.

Conclusions. The results of this study verify the assumption that there are associations between ANS activity and iron metabolism in female subjects. Especially, the changes in functional iron Hb appear to be associated with the changes in HRV. In addition, the finding of concurrent decrements in Hb_{conc} and impaired or unchanged VO_{2max}, leads to assumption that female endurance athletes should react even on small decrements in Hb_{conc}, to avoid the possible performance diminishing effects of impaired functional iron status.

Key words: aerobic capacity, maximal oxygen uptake, heart rate variability, iron status, hemoglobin, serum ferritin, hematocrit, recovery, endurance training.

ABBREVIATIONS

ANS	autonomic nervous system
a-vO_2 difference	arteriovenous oxygen difference
ATP	adenosine triphosphate
Hb_{conc}	hemoglobin concentration
Hb_{mass}	hemoglobin mass
HCT	hematocrit
HF (ms^2)	high frequency variation of R-R intervals (0.15-0.40 Hz)
HR	heart rate
LF (ms^2)	low frequency variation of R-R intervals (0.04-0.15 Hz)
LF/HF	ratio of high to low frequency variation in R-R intervals
NN	normal-to-normal interval
NN50	number of interval differences of successive NN intervals greater than 50ms
pNN50	proportion derived by dividing NN50 by the total number of NN intervals
PNS	parasympathetic nervous system
PSD	power spectral density
R-R interval (ms)	time between adjacent heart beats
RMSSD (ms)	the square root of the mean squared differences of successive R-R intervals, estimate of short-term components of HRV.
SDNN (ms)	standard deviation of R-R intervals, estimate of overall HRV
SNS	sympathetic nervous system
ULF (ms^2)	ultra-low frequency variation of R-R intervals (<0.0033 Hz)
V_E/VO_2	minute ventilation-to-oxygen consumption
V_E/VCO_2	minute ventilation-to-carbon dioxide output
VLF (ms^2)	very low frequency variation of R-R intervals (0.0033-0.04 Hz)
VO_{2max}	maximal oxygen uptake
WHO	World Health Organization

CONTENTS

1 INTRODUCTION

Over the years, athlete monitoring has become a standard practice in helping athletes to reach their peak levels of performance. Because of the numerous different sports and different qualities of athletes even within the sports, studies have pursued to develop different ways and devices to provide individualized information about fitness and recovery status in athletes. The several purposes for athlete monitoring include determining training adaptations and defining training loads, ensuring proper amounts of training and recovery and reducing the risk for sports-related injuries, illnesses and unwanted states of fatigue (Bourdon et al. 2017).

Endurance sports are defined by high volumes of training that occurs at different, often relatively high intensities. Therefore, endurance athletes benefit from athlete monitoring since it helps them to find the balance between sufficient amount of training and recovery. The training in endurance sports aims to enhance aerobic fitness that enables the athlete to work at higher intensities, maintain a certain load for a longer period of time and improve the efficiency of movement. There are many factors that affect aerobic capacity but the most important one of them is probably maximal oxygen uptake, VO_{2max}, that sets the upper limit for the body's capability to deliver and utilize oxygen in the working muscles (Midgley et al. 2007). VO_{2max} is a widely studied and commonly used parameter in monitoring aerobic capacity. It is reliable, has large reference materials and is quite simple to measure from ventilatory gas exchange during maximal exercise.

The monitoring of recovery status is a more complex process since there are many factors that affect recovery and large variation in how different bodily functions react to training-induced stress. One widely used method is detecting the changes in the activity of the autonomic nervous system (ANS), which is highly sensitive to increased amount of stress. ANS responds to stress by altering the activation of its two subsystems: parasympathetic nervous system (PNS) and sympathetic nervous system (SNS), of which PNS activity reacts quickly by decreasing until a sufficient amount of recovery is reached. Heart rate variability (HRV) is closely related to ANS functions and is, therefore, a promising marker of stress and recovery. Endurance training has

both acute and long-term effect on HRV: it is known to decrease acutely after intensive training and this suppression can be observed even at rest. (Hynynen et al. 2007; Martinmäki & Rusko 2008; Plews et al. 2014; Seiler et al. 2007). HRV is also known to rebound to normal levels after a relative resting period (Baumert et al. 2006; Pichot et al. 2000), which makes it an important variable for daily monitoring.

Another valuable method for detecting recovery and homeostasis is monitoring of different iron status variables. Iron is one of the most important micronutrients in the body and is essential for endurance athletes because of its role in oxygen transportation and aerobic energy metabolism. Hemoglobin (Hb), the oxygen transport protein, is responsible for delivering the oxygen to working muscles. Intensive training is known to decrease Hb concentration (Hb_{conc}) and the amount of iron storage protein serum-ferritin (s-Ferr), which helps to maintain Hb count. Studies have found that iron depletion can lead to decreased aerobic performance, especially in female athletes (DellaValle & Haas 2012). Improved iron status might also have long-term effects on aerobic performance since relatively high body weight related Hb_{mass} appears to be critical marker for endurance athletes' future success at the elite national team level (Wherlin & Steiner 2021), underlining the importance of monitoring iron status.

Although several studies have focused on investigating the stress and recovery on professional and recreational athletes, it is not easy to find literature focusing on junior athletes. Monitoring of training is important for juniors since they might not recognize the non-functional overreaching symptoms as easily as professionals who are familiar with high training loads and know their bodies very well. In addition, there is also a lack of longitudinal studies monitoring performance gains and changes in recovery status during one whole training-season with endurance athletes.

Due to these limitations in the literature, the purpose of this study was to examine what kind of changes occur in aerobic capacity and recovery status markers, HRV and iron status, in young female endurance athletes during a six-month training-season. The other purpose was to examine if there are relationships between the changes in aerobic capacity and the two recovery markers or between HRV and iron status, which has not been previously examined.

2 ENDURANCE TRAINING AND PERFORMANCE

The term endurance refers to the ability of the body to sustain certain velocity or power output for the longest possible time (Jones & Carter 2000). Endurance sports are characterized by performances that are prolonged in duration and occur in relatively high intensities, which is why the energy demand during those sport performances is often very high. Most of this energy is produced aerobically in oxidative phosphorylation (Rivera & Brown 2012) making the role of aerobic fitness essential for endurance athletes.

Aerobic fitness can be defined with 4 parameters: maximal oxygen uptake (VO_{2max}), exercise economy, the lactate/ventilatory threshold and oxygen uptake kinematics (Jones & Carter 2000). VO_{2max} is an indicator of the body's capability to deliver and utilize oxygen in the working muscles. Endurance training causes increased VO_{2max} and decreased VO_2 in submaximal intensities due to enhanced oxygen transportation capacity. Exercise economy describes this oxygen uptake required at a certain absolute exercise intensity, and it is not only dependent from the oxygen uptake at a given velocity but also from factors like muscle fiber type and motor unit recruitment, anthropometrics as well as metabolic and technical factors. The lactate/ventilatory threshold, in turn, refers to the intensity at which blood lactate concentration increases from the resting levels with concurrent changes in gas exchange. The improvement of these thresholds, meaning their occurrence on a higher fraction of VO_{2max}, makes it possible to sustain higher absolute and relative intensity without accumulation of blood lactate after training. Finally, the oxygen uptake kinetics, implying to the ability of cardiorespiratory system to adjust to increased oxygen demand, is also enhanced as a result of endurance training and it reduces the oxygen deficit and lactate production at the onset of exercise. (Jones & Carter 2000.)

Enhancements in all the four components of aerobic fitness have been observed to be associated with enhanced endurance performance (Jones & Carter 2000). Training in endurance sports aims for these improvements which makes it possible to maintain the exercise for longer period of time at a certain absolute intensity or to exercise at a higher intensity for a given duration. The training consists of repeated bouts of long durational exercises at different intensities that

improve the function of the metabolic pathways for energy supply (Petibois et al. 2002). Exercises are both aerobic and anaerobic in nature and repeating them many times over a period of time, eventually, results in improved performance in that specific type of exercise. The magnitude of this response is dependent on the duration of the exercise bout, its intensity, frequency and the characteristics of the individual, like training status, gender and genetics. (Jones & Carter 2000.) Training in endurance sports often includes a certain amount of strength training since improved neuromuscular functions are known to enhance the economy of movement and, therefore, performance (Jones & Carter 2000; Midgley et al 2006; Mikkola et al. 2012).

Recovery is an important aspect of exercise training since training and exercise can be regarded as stress factors that disturb the balanced state of body, the homeostasis. When this disturbance is repeated several times, it causes adaptations in which the body aims to become more efficient in that certain type of exercise (Borresen & Lambert 2009). After exercise, the recovery process takes place, and the disturbed homeostasis is restored. Also, most of the exercise-induced adaptations occur during recovery. If recovery after training is insufficient, the adaptations to training and the performance gains can be diminished, and the athlete might drift into a fatigued state. In the worst case, poor recovery can lead to non-functional overreaching or overtraining. (Bishop et al. 2008.)

Cross-country skiing is an endurance sport that requires many different qualities from the athlete since the race distances vary from 1-kilometer to as long as 50-kilometer distances. Today, in addition to traditional interval starts, many of the races are performed with mass starts making speed qualities more important for the athletes (Mero et al. 2016, 491). Strength, especially upper body strength, is also essential for cross-country skiers because of the major role of the double poling technique in classic races. However, similarly to other endurance sports, the most important quality in cross-country skiing is the aerobic fitness and thus aerobic training consists most of the training for cross-country skiers. The training volumes in cross-country skiers are high throughout the year, including large amount of low-intensity aerobic training, weekly high-intensity interval trainings and some amount of speed and strength training. (Gaskill et al. 1999.)

3 PHYSIOLOGICAL ADAPTATIONS TO ENDURANCE TRAINING

Endurance training causes several adaptations in the respiratory, cardiovascular and neuromuscular systems that pursue to enhance aerobic energy production. This occurs due to improvements in oxygen delivery to the working muscles and metabolism in the muscle cells (Jones & Carter 2000). In this chapter, endurance training-induced adaptations in cardiovascular, hematological and autonomic nervous system (ANS) are more closely presented.

3.1 Cardiorespiratory adaptations

Endurance training induces adaptations in the cardiorespiratory system, including different structural and functional changes in heart, vasculature, working muscles and the respiratory system. The structural and morphological changes include changes in the heart size and compliance of the vasculature. The athlete's heart is related to body size but adaptations like increased end-diastolic dimensions in the right and left ventricle, left ventricle hypertrophy and higher left atrium volume and compliance have been presented. (Hellsten & Nyberg 2016; Saltin et al 2000, 226.) Due to the enlargements in heart, training improves diastolic filling and leads to higher end-diastolic heart volumes. Diastolic filling is also enhanced by the improved venous return due to the muscle pump mechanism. The structural adaptations in vasculature include enhanced arterial compliance and increased diameter of the medium and small peripheral arteries. These changes are related to increased oxygen uptake and delivery and improved muscle blood flow perfusion. (Saltin et al. 2000, 225-228.) Endurance training-induced structural changes in the respiratory structures are the strengthening of the respiratory muscles which leads to increased lung volumes (Lazovic et al. 2015.)

Functionally, the most important adaptation to endurance training is the increased maximal cardiac output, the product of heart rate (HR) and stroke volume. This occurs mainly due to increased stroke volume, the volume of blood ejected from the heart within one heartbeat. An improved stroke volume is a result of enhanced cardiac filling which occurs as a consequence of larger cardiac diameter, improved contractility and increased blood volume. (Hellsten &

Nyberg 2016.) The maximal HR does not change as a result of training but it is reduced at rest and in submaximal intensities as a result of alterations in the ANS and sinus node mediation. (Saltin et al. 2000, 230-232.) In peripheral vasculature, endurance training enhances the vasodilation in the capillaries of the working muscles and reduces the sympathetically mediated vasoconstriction, leading to reduced vascular resistance and enhanced muscle blood flow. The improved distribution of blood to working muscles leads to a greater extraction and utilization of oxygen. (Saltin et al. 2000, 225-230.) Endurance training also affects blood pressure by decreasing it at rest and in submaximal intensities which can be partly explained by vascular remodeling, as well as changes in peripheral vascular function and sympathetic nervous system (SNS) activity (Hellsten & Nyberg 2016).

Long-term endurance training adaptations in the functions of the respiratory system include changes in pulmonary ventilation, oxygen-diffusion capacity and arteriovenous oxygen difference (a-vO_2 difference) in blood. In healthy population, the respiratory system is rarely the limiting factor in oxygen delivery, but some adaptations are still required (Guyton & Hall 2011, 1090). Pulmonary ventilation, the flow of air into and out of the lungs, is linearly correlated with oxygen consumption and increases as a response to exercise. The oxygen-diffusion capacity, indicating the rate at which oxygen can diffuse to blood from pulmonary alveoli, increases several-fold during exercise compared to rest, and it is significantly higher in endurance-trained athletes. (Guyton & Hall 2011, 1090-1092.) The a-vO_2 in turn, indicates the difference in O_2 concentration in arterial and venous blood and describes how effectively active muscles extract oxygen for energy production (Rivera & Brown 2012). The capacity of the muscles to utilize oxygen is enhanced as a result of aerobic training which leads to lower oxygen content in venous blood and increased difference in a-vO_2 during both submaximal and maximal intensities (Rivera & Brown 2012).

3.2 Hematological adaptations

Endurance training also induces changes in the composition of blood. It is generally known that endurance training causes expansions in plasma volume with concurrent smaller increases in red blood cell mass resulting in slightly lower hematocrit (HCT) values (O'Toole at al. 1999).

The exercise-induced hypervolemia may have an enhancing effect on oxygen delivery because it decreases the blood viscosity, thereby, improving the blood flow to the working muscles (Rivera & Brown 2012). Larger plasma volume also contributes to the adaptations in cardiac filling and size by increasing venous return. In addition, an increased plasma and blood volume enhances the thermoregulation of body. (Hellsten & Nyberg 2016.)

The effect of hypervolemia is two-sided: even though the increased plasma volume enhances the blood flow in the working muscles and the efficiency of thermoregulation, low HCT limits the amount of oxygen carrying proteins per unit of blood (O'Toole et al. 1999). Hemoglobin (Hb), a component of red blood cell structure, is the main oxygen carrying protein in blood and its concentration is commonly measured in both clinical and exercise physiological studies. An increased plasma volume results into lower Hb concentration (Hb_{conc}) (Rivera & Brown 2012) and severe reductions in Hb limits the oxygen carrying capacity in blood which might lead to lower VO_{2max} values and decreased performance (Weaver & Rajaram 1992). However, only small decreases in Hb_{conc}, staying within normal reference values, should not limit endurance performance because the increased cardiac output can maintain the adequate delivery of oxygen carrying proteins to the working muscles (Schmidt & Prommer 2010).

3.3 Adaptations of the autonomic nervous system

Autonomic nervous system (ANS) is involved in the regulation of many bodily functions, including regulation of the cardiovascular system. The system consists of two divisions: parasympathetic nervous system (PNS) and sympathetic nervous system (SNS) which both have inhibitory and excitatory effects on their end organs. The effects of the subsystems are often reciprocal with each other (Guyton & Hall 2011, 778): the vagal activity (the activity of PNS) affects cardiac pumping by decreasing the sinus rhythm and HR, while SNS has an opposing effect on the heart and increases HR and the force of contraction of the cardiac musculature. (Guyton & Hall 2011, 129.) Other cardiovascular functions, including blood pressure and peripheral vascular tone are also affected by ANS (Saltin et al. 2000, 225-228).

ANS activity reacts acutely to exercise by increasing the SNS and decreasing the PNS activity as a response to increased blood pressure and venous return at the onset of exercise (Michael et al. 2017). During recovery, the control of cardiac regulation shifts back under PNS control. Several other mechanisms, such as muscle mechanoreceptors and baroreceptors, also take part into the contribution of changes in cardiac pumping during exercise (Saltin et al. 2000, 232-235).

The long-term adaptations of the ANS to endurance exercise training include an increased vagal tone to the heart at rest and during submaximal exercises. The suggested explanatory mechanism is that the cardiac muscle receptor activity is altered in the cardiac muscle which results into modulation of ANS activity (Saltin et al 2000, 232-235). For example, the activation of cardiac baroreceptors is increased in response to enlarged blood and stroke volume, which leads to enhanced PNS activity and reduces the sympathetic influence. Also, the upregulation of the dopaminergic receptors and downregulation of enkephalin receptors in the cardiac muscle enhance the effect of PNS activity leading to increased vagal influence on the heart at rest and during submaximal exercise (Saltin et al. 2000, 232-235).

As presented in this chapter, endurance training causes many acute changes and long-term adaptations in bodily structures and functions. These adaptations are not limited only to the adaptations of here discussed systems: essential adaptations also occur in the metabolic pathways and in the composition of the working muscles. Table 1 summarizes the expected adaptations that studies have observed to follow long-term endurance training.

TABLE 1. Summary of expected adaptations to long-term endurance training. (Guyton & Hall 2011, 1085-1095; Hellsten & Nyberg 2016.)

	Variable	Effect
Cardiovascular	Heart size	↑
	Stroke volume	↑
	Heart rate	↓ at rest and submaximal intensity
	Cardiac output	↑ at maximal intensity
	Blood flow	↑
	Systolic blood pressure	↓ at submaximal, ↑ at maximal intensities
Hematological	Plasma volume	↑
	Red blood cell mass	↑
	Hemoglobin mass	↑
	Hemoglobin concentration	↓
Respiratory	Pulmonary ventilation	↓ at submaximal, ↑ at maximal intensities
	Pulmonary diffusion	↑ at maximal intensity
	a-vO_2 difference	↑
	Oxygen consumption	↓ at submaximal, ↑ at maximal intensity
Neural	PNS activity	↑ at rest and at submaximal intensity
Muscular	Type I muscle fiber size	↑
	Capillary density	↑
	Myoglobin content	↑
	Mitochondrial number and size	↑
	The number of oxidative enzymes	↑
Metabolic	Lactate threshold	↑
	Respiratory exchange ratio	↓
	Oxygen consumption	↓ at submaximal, ↑ at maximal intensity

↑, increase; ↓, decrease; a-vO_2 difference, arteriovenous oxygen difference.

4 MONITORING ENDURANCE PERFORMANCE WITH AEROBIC CAPACITY

Monitoring of endurance performance is important for athletes to detect the training-induced improvements and to find out which qualities still require future enhancements. Endurance performance is affected by numerous factors which partly depend on the type of endurance sport, but the key determinant is aerobic capacity, the ability to sustain high work rates. As already discussed in chapter 2, aerobic capacity is affected by four factors: maximal oxygen uptake (VO_{2max}), exercise economy, the lactate/ventilatory threshold and oxygen uptake kinematics. From these factors, VO_{2max} is probably the most widely used parameter in monitoring aerobic capacity because of its close relations to endurance performance.

4.1 Maximal oxygen uptake

VO_{2max} describes the maximal rate of oxygen consumption and utilization during exercise (Basset & Howley 1999). The parameter has established its status as one of the most used parameters in aerobic exercise testing ever since it was discovered in the 1920s. Back then, Hill and Lupton (1923) created four basic assumptions according to VO_2: 1st, there is an upper limit to oxygen uptake, 2nd, there are interindividual differences in VO_{2max}, 3rd, a high VO_{2max} is a prerequisite for success in middle and long-distance running and 4th, VO_{2max} is limited by ability of the cardiorespiratory system to transport O_2 to the muscles (Basset & Howley 1999). Today, these assumptions still take place and the use of VO_{2max} has widened its usage among many different endurance sports and clinical testing.

VO_2 is a product of cardiac output (Q) and a-vO_2 difference (Equation 1). Cardiac output is a product of HR and stroke volume while the a-vO_2 difference refers to the O_2 difference in arterial and venous blood, indicating the effectiveness of the active muscles in extracting oxygen from arterial blood. During exercise, VO_2 increases to meet the demand of oxygen in the working muscles until it reaches the maximum level, VO_{2max}. The capacity of the cardiorespiratory system to deliver oxygen to the working muscles has widely been accepted to limit the VO_{2max} rather than the muscles' ability to utilize it. Basset and Howley (1999) separated the possible limiting factors of the cardiorespiratory system into two categories: the

central factors, that include the pulmonary diffusion capacity, maximal cardiac output and oxygen carrying capacity of the blood, and the peripheral factors, meaning the skeletal muscle characteristics. According to present literature, the maximal cardiac output seems to be the main limiting factor for VO_{2max} (Midgley et al. 2007).

$$VO_{2max} = Q \times (C_aO_2 - C_vO_2)$$

EQUATION 1. Definition of maximal oxygen uptake (VO_{2max}) defined by Fick equation. Q, cardiac output; C_aO_2, the arterial oxygen content; C_vO_2, the venous oxygen content.

The VO_{2max} measurements are executed with incremental exercise tests that usually include 7-12 two-to-three-minute workloads and end to exhaustion. The VO_{2max} is measured from the ventilatory gas exchange by defining the ventilation and the concentration of inhaled and exhaled oxygen and carbon dioxide (Mero et al. 2016, 290-293). VO_2 increases in response to increased energy and oxygen demand in the working muscles and it is affected by the training status of the individual. Normal values at rest for a young man are around 250 ml/min but under maximal conditions it can increase to values from 3.6 l/min in untrained man to over 5.1 l/min in elite endurance trained athlete (Guyton & Hall 2011, 1090).

In addition to training status, VO_{2max} is affected by other factors like age, sex, and genes. Both longitudinal and cross-sectional studies have observed age-associated changes in VO_{2max} and found progressive regressions in VO_{2max} around the age of 30 (Fleg et al. 2005; Hawkins et al 2003). The reductions are related to changes in physical activity and body composition and in athletic individuals, the cessation in VO_{2max} is noted to occur non-linearly upon decrement in training (Hawkins et al 2003). The VO_{2max} values are higher in men than women, for example, Helgerud et al. (1994) observed about 10 % (23 ml/kg/min) higher VO_{2max} values and higher VO_2 values on the same absolute running speed in men than in women with similar performance levels. The difference between sexes can be largely explained by the difference in body composition (Helgerud et al. 1994; Latin et al. 1997). Genetics are also an important determinant for VO_{2max} since maximal heritability, when adjusted to age, sex and body mass

can reach about 50 % of the residual variance (Bouchard et al. 1998). The VO_{2max} response to training reaches a maximal heritability estimate of 47 % (Bouchard et al. 1999).

4.2 Maximal oxygen uptake and endurance performance

VO_{2max} sets up an upper limit for endurance performance since exercising above that threshold cannot be maintained for extended periods (Midgley et al. 2007). Therefore, VO_{2max} is highly associated with endurance performance and endurance-trained athletes generally show significantly higher VO_{2max} values that can be even two-folded compared to sedentary individuals (Guyton & Hall 2011, 1090). These higher values are caused by the enhanced stroke volume, improved myocardial function and higher oxidative capacity in the working muscles that enhance the delivery and utilization of oxygen in the working muscles (Midgley et al 2006).

Studies have shown that endurance training can cause increments in VO_{2max} even within short periods of time in recreational and sedentary subjects. Vesterinen et al. (2013) found significant improvements in VO_{2max} in recreational endurance runners in response to 14 weeks of basic low-intensity aerobic training. In the study, the training regimen continued for another 14 weeks with higher training volumes and intensity but although VO_{2max} still increased, it was not considered significant. Carter et al. (1999) also found significant improvements (9.9 %) in VO_{2max} in physically active but not highly-trained population in response to only six-week training regimen.

The effect of exercise intensity on the VO_{2max} enhancements has been observed to play a critical role, especially, in elite athletes. In sedentary individuals, regular and even short-term aerobic training has been observed to enhance VO_{2max} values. For example, Tabata et al. (1996) found that only six weeks of endurance training on young physically active males at 70 % of VO_{2max} caused significant improvements in VO_{2max}. Another training group in their study did high intensity interval training for the same amount of time and the improvements in aerobic capacity were similar with that of the traditional aerobic training group. For already endurance trained athletes, the improvement of VO_{2max} is not as simple, and it has been suggested that the VO_{2max} reaches a plateau after several years of training (Midgley et al. 2006). However, some studies

have observed enhancements in VO_{2max} in elite athletes as a response to high-intensity interval training. For example, Ní Chéilleachair et al. (2017) observed significantly larger improvements in VO_{2max} and 2000 m time-trial performance in trained rowers after eight-week high intensity interval training compared to long slow distance training.

Several studies have shown that in endurance-trained athletes, performance can be enhanced also without concurrent improvements in VO_{2max} (Legaz et al. 2005; Lindsay et al. 1996). VO_{2max} is not the only determinant for aerobic performance and therefore, performance gains can be obtained by enhancing other aerobic fitness parameters such as exercise economy, the lactate/ventilatory threshold and oxygen uptake kinematics (Jones & Carter 2000). However, VO_{2max} is an important factor for endurance performance because it limits the VO_2 that can be sustained and where the actual performance is committed (Midgley et al.2012; Midgley et al. 2006). Improving VO_{2max} increases the possible sustainable fraction of VO_{2max}, leading to enhanced performance.

5 MONITORING TRAINING STATUS AND RECOVERY

In endurance sports, training includes high training volume, intensity and frequency of training throughout the year. These constantly high training loads increase the risk of reaching the levels of non-functional overreaching, overtraining and the likelihood of developing sports-related injuries which is why it is important for the athletes to program their training and recovery to optimize their development and to avoid the unwanted states of fatigue.

The monitoring of training and recovery status has been established in all sorts of sports to quantify the load of training and to optimize fitness and performance enhancement. For this purpose, several indicators and physiological parameters have been designed. Different heart rate variables, such as simple HR measurements during training as well as recovery and heart rate variability measurements, are commonly used since they are easily obtained and predict training and recovery status quite reliably (Djaoui et al. 2017). Especially, heart rate variability (HRV) has established its status as a common exercise recovery marker, because it has been noted to adapt to training and to measure sensitively the level of autonomic control (Borresen & Lambert 2008).

Studies have also pursued to assess changes in several biochemical markers in blood to quantify training load and the training-related changes in body homeostasis. Measurements related to body iron status, like hemoglobin and plasma ferritin, are commonly used, since reductions in body iron status are known to affect athletic performance (Djaoui et al. 2017). Iron status is also known to be highly related to aerobic capacity and VO_{2max}, which makes it potential indicator of endurance capacity (Hinrichs et al. 2010.) In this chapter, the two physiological markers of training status, HRV and iron status biomarkers, will be presented.

5.1 Heart rate variability

HRV is a widely used parameter in estimating cardiac autonomic regulation. The term refers to the fluctuations in time intervals between two consecutive heart beats. Usually, a heartbeat interval is defined as the time between adjacent R wave peaks (Pumplra et al. 2002) that

demonstrate the depolarization of the ventricles of the heart. These R waves can be detected via electrocardiogram (ECG) as shown in figure 1.

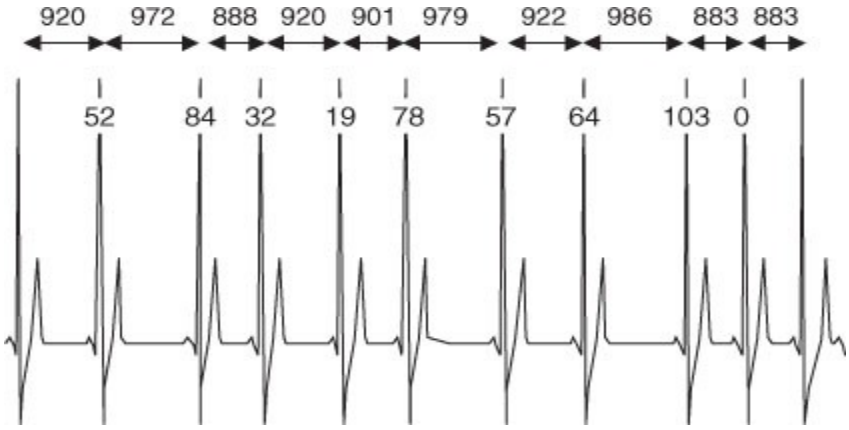


FIGURE 1. ECG output over 11 beats with R-R interval times and difference between adjacent R-R intervals (Achten & Jeukendrup 2003).

HRV is a product of heart-brain interactions and it reflects changes in efferent activity of the autonomic nervous system (Shaffer & Ginsberg 2017). The central origin of the cardiovascular regulation system locates in medulla oblongata, where the sensory information from higher brain centers and afferent feedback from peripheral receptors is integrated. According to this information, the cardiovascular center adjusts cardiac functions by changing the efferent activity of the parasympathetic and sympathetic nervous system, the two subsystems of ANS (Shaffer et al. 2014.) These subsystems influence heart rate by modulating the intrinsic firing rate of the heart's pacemaker cells in sinoatrial and atrioventricular nodes (Pumprla et al. 2002). Both PNS and SNS also innervate the atrial and ventricular muscle cells.

The effects of PNS and SNS on cardiac modulation are converse: vagal modulation decreases HR and increases the variation in the cardiac rhythm while SNS increases HR and decreases HRV (Pumplra et al. 2002.) Understandably, parasympathetic efferent activity is higher at rest and sympathetic efferent activity dominates during exercise. The different effects of PNS and SNS on cardiac modulation are most likely caused by the different neurotransmitters released from their nerve synapses. The PNS nerve synapses release acetylcholine which has a very short

latency period and a high rate of turnover which causes rapid responses in the regulation of cardiac rhythm. Therefore, PNS can regulate the heart in beat-to-beat basis. In turn, SNS mediates the heart with the synaptic release of noradrenaline of which reabsorption and metabolization occurs in slower manner, causing the alterations in cardiac functions to take place with a delay. Due to the different neurotransmitter functions, the two autonomic divisions operate at different frequencies, making it possible to identify and quantify the differences in the activity of PNS and SNS. (Pumprla et al. 2002).

5.1.1 Methods for analyzing heart rate variability

HRV measurements include several steps, starting with ECG recording and detection of all the QRS complexes and R-R intervals. After this, the signal must be processed: the recorded data is first transferred into digital form, then the artifacts are deleted and eventually the R-R intervals are converted to N-N intervals, the intervals between successive normal intervals. From this processed data HRV can be analyzed. (Task Force 1996.) The two most commonly used methods in analyzing HRV are the time-domain analysis and the frequency domain analysis, also known as power spectral density analysis (PSD). Both analyzing processes include several parameters that represent changes in the activity of PNS and SNS.

Time domain measurements. Time domain indices are probably the easiest HRV parameters to obtain, since they are simply computed by using statistical measures (Aubert et al. 2003; Shaffer et al. 2014). They are also comparable variables, presuming, that the calculations are made from epochs of the same length. The limitation of these indices is their incapability to distinct the activity of the PNS and SNS and to determine the rhythmic activity generated by the different physiological control systems. (Aubert et al. 2003; Shaffer et al. 2014).

The most commonly used time domain variables are probably SDNN, the standard deviation of NN intervals and RMSSD, the square root of the mean squared differences of successive NN intervals. SDNN is affected by both SNS and PNS activity, and it is highly correlated with frequency domain variables (Shaffer & Ginsberg 2017). SDNN is dependent from the length of the analyzation period, which makes it important to standardize the duration of recording (Task

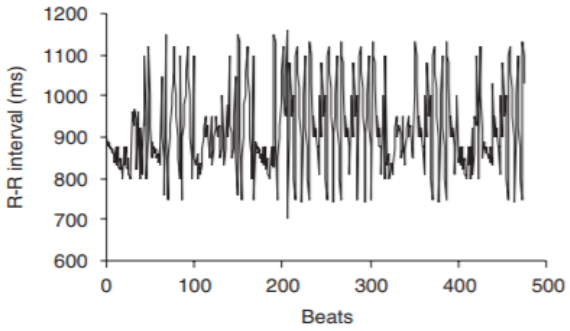
Force 1996). In short term recordings, SDNN in mainly an influence of PNS mediated respiratory sinus arrythmia (RSA) (Shafferet al. 2014). RMSSD measurements require more data processing, since the calculated successive time differences between heartbeats are first squared and the results averaged after which the square root of the total is obtained. RMSSD indicates beat-to-beat fluctuations in HR, and it reflects PNS mediated changes in HRV. The variable correlates with frequency domain variable, HF, which is also an indicator of PNS activity. (Shaffer et al. 2014.)

There are also other commonly used time domain variables like SDNN derived SDANN and SDNN-index that are 5-minute segments from the total SDNN measurement and pNN50 which is the percentage of adjacent NN intervals that differ from each other by more than 50 ms (Shaffer et al. 2014; Task Force 1996). These variables are often measured alongside SDNN and RMSSD but they do not necessarily provide more information, which is why the two main measures are more recommendable (Shaffer et al. 2014). An example from the time domain analysis and the most used variables are shown in the figure 2.

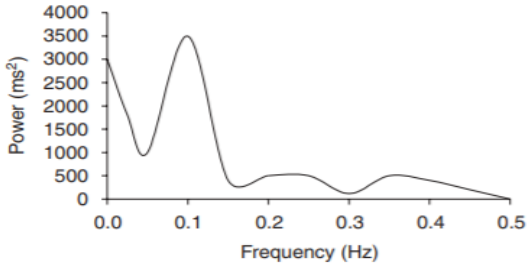
Frequency domain measurements. In this analyzation process, NN data sequence is interpolated and then separated into component rhythms that operate at different frequencies. The method measures how the variance and amplitude of a given rhythm (power) is distributed as a function of frequency (certain time scale of a given rhythm). (Shaffer et al. 2014.) The power can be calculated by using parametric and non-parametric methods, which both provide comparable results (Task Force 1996). The values are presented as power spectral density, which is the area under curve in a given segment of the spectrum. The recording lengths should also be strictly standardized in this analyzation method, since it has a large effect on the variable. (Shaffer et al. 2014.)

The recorded data in frequency domain analysis is divided into different frequency bands that all give information about the activity of PNS and SNS. The high-frequency (HF) power spectrum describes the amount of HRV occurring at frequencies between 0.15 – 0.4 Hz and is used to describe vagal activity. HF also corresponds to the HRV associated with the respiratory cycle. The second frequency band is called low frequency power spectrum (LF) that ranges

between 0.04 and 0.15 Hz. LF power has been observed to describe vagal activity, primarily baroreceptor activity. There is also conflicting evidence that the LF band would also reflect SNS activity and due to this, some studies have used the LF/HF ratio as an indicator of the balance between SNS and PNS activity. The last two frequency bands in HRV analyses are very-low-frequency (VLF) and ultra-low-frequency (ULF) bands that occur in 0.0033 – 0.04 Hz and under 0.0033 Hz. (Shaffer et al. 2014.) VLF rhythm is intrinsically generated by the heart and is affected by the efferent activity of SNS. ULF in turn is caused by the circadian rhythm of HR but the contribution of ANS divisions efferent activity to this band is still unknown. (Shaffer & Ginsberg 2017.) Figure 2 represents R-R intervals in frequency domain analysis and the different frequency bands used to evaluate ANS activity.



R-R intervals in time-domain	
AverageNN (ms)	Average of all normal R-R intervals
SDNN (ms)	Standard deviation of all normal R-R intervals
r-MSSD (ms)	Root mean square successive difference
pNN-50 index (%)	Percentage of differences between adjacent normal R-R intervals that are >50ms



R-R intervals in frequency-domain	
Total power (ms^2)	The power in the heart rate power spectrum between 0.00066 and 0.34Hz
VLFP (ms^2)	The power in the heart rate power spectrum between 0.0033 and 0.04Hz
LFP (ms^2)	The power in the heart rate power spectrum between 0.04 and 0.15Hz
HFP (ms^2)	The power in the heart rate power spectrum between 0.15 and 0.36Hz
LFP : HFP ratio	

FIGURE 2. Examples of the time-domain and frequency domain analyzes. On the left, a graph of R-R interval time between each subsequent beat measured over a 7-minute period at rest (~500 beats) and common ways to express heart rate variability in the time-domain are presented. The graph on the right is an example of the power spectrum showing the magnitude of the variability as a function of frequency. The most commonly found areas in the power spectrum, which represent different influences of the sympathetic and parasympathetic nervous systems, are displayed in the box below the right figure. (Achten & Jeukendrup 2003.)

5.1.2 Factors affecting heart rate variability

HRV is affected by numerous factors including neuropsychological, environmental, physiological and pathological, lifestyle, and non-modifiable factors (Fatisson et al. 2016). In the field of exercise sciences, the most essential factors are the ones related to physical activity and to the characteristics of the individual like age, gender, and perceived psychological stress.

Age and gender are non-modifiable factors that have significant effects on HRV. With age, HRV is known to decrease, which might be related to changes in ANS functions (Carter et al. 2003; Fatisson et al. 2016). These changes include reductions in the PNS control of the heart and reduced response of cardiac muscle to sympathetic activation. The effect of gender on HRV is not as clear but it seems that women tend to have higher parasympathetic control and lower sympathetic control of the heart. For example, Hedelin et al. (2000) examined young endurance trained cross-country skiers and found that women had higher HF and total HRV values than men. HF power is known to be an indicator of parasympathetic activity which underlines the assumption of higher parasympathetic control on women. Jensen-Urstad et al. (1997) observed untrained healthy subjects and noted women to have lower LF, LF/HF, VLF and total power that are main indicators of SNS activity. Similar findings on women's parasympathetic dominance and men's sympathetic dominance were also observed by Evans et al. (2001).

Stress is generally known to affect ANS activity, whether it was psychologically or physically generated. Psychological stress and negative emotions lower the PNS activity and cause decreased HRV values (Fatisson et al. 2016; Kim et al. 2018; Michels et al. 2013). The effect of exercise training induced stress on HRV is more widely studied and acutely, exercising is known to cause decreased HRV values. Chronic exercise training, in turn, increases the vagal modulation of the heart and HRV and can reduce the decrement in HRV that occurs with aging (Achten & Jeukendrup 2003: Carter et al. 2003). Since low HRV is known to be associated with mortality, it can be concluded that physical activity could serve as a valuable tool in maintaining cardiac health.

In addition, several studies have observed that cardiac functions are also affected by the circadian rhythm (Carrington et al. 2003; Furlan et al. 1990). Furlan et al. (1990) committed a 24-hour recording from HRV and found that daytime was associated with relative sympathetic dominance while vagal activity was more present during the night. Carrington et al. (2003), in turn, observed night-time decreases in HR, blood pressure and LF/HF component of frequency domain analysis, which reflects a greater contribution to sympatho-vagal balance. They also observed increases in the HF component during the night, emphasizing the role of vagal influence. Despite the large variety of studies, sleep and circadian rhythm-induced changes in HR, blood pressure and ANS activity around the time of sleep onset are still not fully understood. (Carrington et al. 2003.)

5.1.3 Heart rate variability and endurance performance

HRV is widely used as a parameter of recovery in exercise physiology because of its close relations to ANS functions. Since vagal activity is known to increase as a response to improved endurance performance and decrease during stress, the measurements of its activity can be used to evaluate the athletes' recovery status and adaptations to training.

HRV parameters react acutely to exercise by decreasing at the onset of exercise, after which they return to resting levels during recovery, reflecting the expected changes in ANS activity (Michael et al. 2017). The decrement in HRV seems to be dependent on the exercise intensity and, for example, Tulppo et al. (1998) observed incremental decreases in the HF component after the onset of exercise as a function of exercise intensity. Similar findings from the effect of exercise intensity on HRV were also found in other studies (Pichon et al. 2004; Saboul et al 2015). The effect of exercise duration and volume on HRV is not as clear. For example, Saboul et al. (2015) failed to find any exercise duration-related changes in HRV in response to exercise, while Pichon et al. (2004) noted notably greater decreases in the HF and LF components during exercise with longer duration of training. A possible explanation for the different result can be the different measurement designs but more research on this field is still needed.

HRV recovery after exercise also depends on the intensity of the exercise. Seiler et al. (2007) studied endurance-trained athletes and noted that after training at low intensities, HRV returned to resting levels in 5-10 minutes after the exercise session. After the exercise above the ventilatory threshold (the intensity at which an increase in V_E/VO_2 occurs without an increase in V_E/VCO_2), a significant delay in HRV recovery was observed and the effect was higher on less trained athletes. (Seiler et al. 2007.) Similar findings were obtained by Martinmäki & Rusko (2008), concluding that the higher metabolic demand during exercise slows down the restoration of autonomic control of the heart. The effect of training on cardiac autonomic modulation can also be observed after a longer period by using nocturnal HRV. For example, Myllymäki et al. (2012) found significantly lower RMSSD values during the night after longer 90-minute exercise compared to a control night, while Hynynen et al. (2010) found clear decreases in SDNN, RMSSD, and HF after heavy marathon training and moderate exercise session.

As discussed in the earlier chapters, long-term endurance training causes adaptations in the ANS activity and these adaptations can be seen in HRV in rest and recovery. At rest, HRV increases indicating greater parasympathetic activity and lower sympathetic activity, thus contributing to training induced bradycardia (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013). Endurance-trained athletes have also been observed to recover faster from the reduced vagal outflow than untrained individuals (Hautala 2001; Stanley 2013). Similarly, Tulppo et al. (1998) stated that better physical fitness leads to smaller decrease in HRV during exercise at submaximal intensities.

Although chronic endurance training seems to increase the proportion of vagal modulation of cardiac events, cumulative effects of heavy endurance training have been established to decrease HRV thus indicating suppressed parasympathetic activity (Baumert et al. 2006; Hynynen et al. 2007; Pichot et al. 2000; Plews et al. 2014). Plews et al. (2014) examined the relationships between HRV and training intensity distribution in international level rowers and observed suppressions in parasympathetic activity after training periods including a great amount of high-intensity training. Similar results were found by Hynynen et al. (2007) who reported decrements in nocturnal HRV after an overreaching period in elite cross-country skiers. Pichot et al. (2000) noted that the decrement in HRV occurs progressively during an

intensive training period and that HRV rebounds during relative resting week in middle distance runners during their usual training cycle. This rebounding effect of HRV was also observed by Baumert et al. (2006) on rest days, following intensified training in track and field and triathlon athletes.

Since HRV has been found to be related to exercise-induced fatigue and recovery from it, numerous studies have investigated if HRV could be used to monitor training and avoid excessive fatigue. Following the changes in resting HRV could be beneficial, since for example Schmitt et al. (2008) found clear association between individual changes in resting HRV (measured in supine position) and changes in aerobic capacity (VO_2 at ventilatory threshold) in elite cross-country skiers and biathletes after 12 weeks of training in altitude. In addition, HRV-guided training has produced positive results on physical performance and training adaptations (Kiviniemi et al. 2007; Vesterinen et al. 2016). Vesterinen et al. (2016) examined how HRV-guided training affected VO_{2max} and 3000 m running performance on recreational endurance runners. Subjects were divided into two training groups with one group following a predefined training program and the other group (HRV-guided group) following moderate and high intensity trainings based on individual HRV measurements. Significant difference was found between the groups, since the 3000 m performance was improved for the HRV-guided group but not for the other group. However, VO_{2max} was improved in both groups. (Vesterinen et al. 2016.) Kiviniemi et al. (2007) ended up with similar results with moderately fit males, showing significantly greater enhancement in maximal running speed for HRV-guided training group compared to predefined training group, while no between group differences were found in VO_{2max}.

Non-functional overreaching and overtraining have usually been observed to be associated with reduced HRV. Kajaia et al. (2017) found significantly lower values in time-domain parameters (mean R-R, SDNN, RMS-SD, pNN50) and in the HF component of spectral analysis on athletes suffering from overreaching or overtraining reflecting lower variation in HR and lower vagal influence on cardiovascular function. In the study, the LF and LF/HF ratio values were significantly higher on overreached or overtrained athletes, which indicates increased sympathetic activity. (Kajaia et al. 2017.) Lower HRV values were also found in studies

detecting overtrained cross-country skiers (Hynynen et al. 2007) and triathletes (Plews et al. 2012).

Despite most studies showing strong relationships between reduced HRV and fatigued state of an athlete as well as increased HRV and an enhanced performance, many studies have observed diverse results. An increased HRV is not always associated with better aerobic capacity (Achten & Jeukendrup 2003) and some have even showed associations between decreased performance and unchanged or increased HRV. These divergent findings can be explained due to the methodological approaches adopted, difficulty with defining the overtraining state and the possibility that two types of overtraining (parasympathetic and sympathetic) may occur in athletes. (Plews et al. 2013.) However, because some studies have shown these different findings about HRV and its relationship with fatigue, the interpretation of HRV should be always made with caution.

5.2 Iron status

Iron is one of the most important micronutrients in body and it has an important role in oxygen transportation and aerobic energy metabolism in the electron transport chain (Cook et al.1992). Body iron can be divided into three categories due to its functions: storage iron, transport iron and functional iron. Storage iron is located, for example, in bone marrow, where the red blood cell production occurs. A small fraction of storage iron can also be found in blood bound to ferritin protein. Transport iron locates in blood bound to transferrin molecule and its function is to transport iron to tissues for erythropoiesis. Functional iron refers to the iron that is available for tissues, practically meaning the oxygen carrying iron bound to hemoglobin molecule. (Pfeiffer & Looker 2017.)

In iron depletion, body iron stores are insufficient to meet the metabolic demands of the body, leading to iron deficient erythropoiesis and eventually to iron deficit anemia. (Garcia-Casal et al. 2018). Iron depletion occurs gradually starting from the depletion of storage iron, after which the amount of transport iron and finally the content of functional iron is depleted (Figure 3). Low iron values have many negative influences in body, including fatigue, impaired working

ability and decreased physical performance (Garcia-Casal et al. 2018) which is why maintaining adequate iron status is important for overall health. As a result, studies have pursued to develop different methods to determine the body iron status.

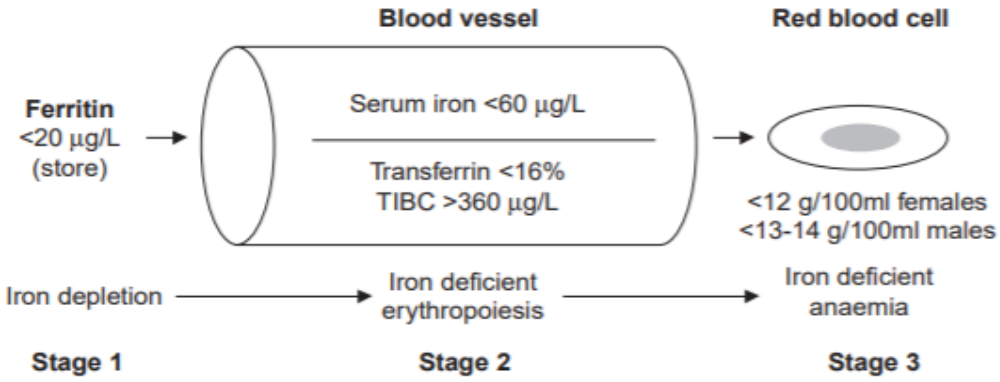


FIGURE 3. Body iron types and the development of iron deficient anemia (Chatard et al. 1999).

5.2.1 Iron status parameters

The assessment of body iron status from bone marrow biopsies has been regarded as the golden standard for the diagnosis of iron deficiency (Garcia-Casal et al. 2018). However, it is an expensive and invasive method, which is why studies have pursued to develop more simple laboratory methods for iron status assessment. There are many iron status parameters for different iron types, for example serum ferritin that is used in the evaluation of storage iron content. By measuring the changes in the concentration of different iron types, it is possible to provide information about the current iron status and about the possible development and the state of iron deficiency. In this chapter, commonly used iron status markers serum ferritin, hematocrit and hemoglobin are more closely presented.

Serum ferritin. Ferritin is the main storage iron-binding protein, and it can be found from many tissues in body. Only a small fraction of ferritin can be found in plasma, usually 12-300 µg/l,

and it has been noted to reflect the amount of whole-body iron quite reliably in healthy subjects (Cook ym. 1992, WHO 2011). For example, Garcia-Casal et al. (2018) examined the reliability of serum ferritin to measure iron deficiency and iron excess compared to bone marrow and liver biopsies. In the reviewed nine studies, serum ferritin during iron deficiency in otherwise healthy subjects was 12-18 ug/l, which is quite similar to the 15 ug/l reference value defined by World Health Organization (WHO 2011). The strengths of this iron status measure are the international reference materials and the strictly defined cut-off values which make the results comparable between studies (Pfeiffer & Locker 2017). The reference values are also designed specifically for different age groups, sexes and for women during pregnancy, since all these factors have an effect on serum ferritin levels (Cook ym. 1992; Rocha ym. 2008).

There is a one important limitation in the use of serum ferritin: ferritin is an acute-phase protein which reacts to inflammation by increasing the serum ferritin concentration (WHO 2011). In Garcia-Casal's et al. (2018) review, the average value for serum ferritin on subjects suffering from different chronic inflammatory diseases was 83,43 ug/l during iron deficiency and the variability between studies was large. The incapability to define certain cut-off values for serum ferritin on people suffering from inflammation makes it impossible to identify iron status by using only this parameter. The other limitation with serum ferritin is that it cannot be used to evaluate the severity of iron deficiency. The parameter is a valid method in assessing iron deficiency until the concentration reaches the reference value 15 µg/l. After this, the storage iron levels are known to be too low, but the severity of iron deficiency remains unknown (Pfeiffer & Looker 2017). Therefore, in order to evaluate iron status during inflammation and its deleteriousness, it is recommended to measure other iron status parameters simultaneously with serum ferritin.

Hemoglobin. Hb is the oxygen binding protein in red blood cells. It is usually measured as the concentration in blood, Hb_{conc}, and it is one of the most common iron status indicators that reflects the levels of functional iron and it is fast, cheap and simple variable to measure (Mei et al. 2005). Similarly to serum ferritin, Hb_{conc} has strictly standardized international reference materials that make the measurements valid and comparable between studies (Anderson & McLaren 2012, 501). Hb_{conc} lacks in the capability to observe the development of iron deficiency, since the values change only after the homeostasis between iron depletion and

supply has been unequal for a while and the iron storages are already significantly depleted (Mei et al. 2005). However, after iron deficiency is present, the variable can be used to evaluate the severity of iron deficiency since the values drop as the iron is more depleted (Pfeiffer & Looker 2017). Other disadvantages with Hb_{conc} measurements are that it is affected by quite many factors, like inflammatory reactions, pregnancy, smoking, altitude and dehydration. (Pfeiffer & Looker 2017.)

Hematocrit. Along with hemoglobin, HCT is one of the most common parameters obtained in laboratory measurements (Cook et al. 1992). Hematocrit is a functional iron parameter, and it reflects the percentage of solid material in blood, basically meaning the portion on blood cells. Because red blood cells make up most of the cells in blood, hematocrit is thought to reflect the mass, amount and volume of the red blood cells. The strengths of this variable are the international reference values made for the variable and that it is easily obtained. However, hematocrit is an unspecific variable that does not tell if red blood cell production is really lacking from iron. Also, the changes in HCT appear only after iron deficiency is already present since the regeneration of red blood cells in blood lasts for several days, meaning that the iron deficient erythropoiesis has been present for a while before the changes in HCT values can be detected. (Cook et al. 1992; Anderson & McLaren 2012, 252-253).

In conclusion, serum ferritin can be recommended for iron status assessment in the detection of iron deficiency in healthy subjects. However, it does not reflect the severity of iron deficiency, which is why it is recommendable to simultaneously measure the markers of functional iron like Hb_{conc} and HCT. Especially, the combination of serum ferritin and Hb_{conc} provides an encompassing picture of whole-body iron status. For example, Mei et al. (2005) stated that it has the best diagnostic efficiency in iron status assessment. Summary of the strengths, limitations and the current cut-off values of these three iron status parameters are presented in table 2.

TABLE 2. A summary of the strengths, limitations and current cut-off values for serum ferritin, hematocrit and hemoglobin (Modified from the table of Anderson & McLaren 2012, 253.)

Variable	Strengths	Limitations	Cut-off values
Serum ferritin	Describes the status of storage iron, standardized reference materials	Acute-phase protein	<12-15 µg/l
Hematocrit	Easy, fast, cheap, standardized reference materials	Reacts slowly, Non-specific, Affected by factors not related to body iron	M: 39–50 % F: 35–46 %
Hemoglobin	Easy, fast, cheap, standardized reference materials	Reacts slowly, Non-specific, Affected by factors not related to body iron	M <130 g/l, N <120 g/l

5.2.2 Iron status and endurance performance

Due to iron's essential roles in energy metabolism, it is a very important nutrient for athletes, especially for endurance athletes. Maximum oxygen uptake, the product of cardiac output and arteriovenous oxygen difference, is the key determinant of endurance capacity. Oxygen transportation in blood, one of the two components affecting arteriovenous difference and transport capacity, depends on the availability of the oxygen transport protein hemoglobin. (Hinrichs et al. 2010.) Consequently, adequate hemoglobin concentration has a significant effect on endurance performance. Furthermore, maintaining transport and storage iron homeostasis is important because their depletion causes a decrement in hemoglobin count.

Intensive training increases iron depletion, which is why endurance athletes should pay attention to sufficient iron supply (Ostojic & Ahmetovic 2008). Iron depletion is mainly caused by increased energy demand (Ostojic & Ahmetovic 2008) but during exercise, iron is also

excessed from the body due to sweating, hematuria and bleeding of the digestive system (Chartad et al. 1999). Especially female endurance athletes, who lose blood and iron also during their menstruation cycle, should pay attention to maintaining iron status.

Studies have shown clear associations between aerobic capacity and functional iron status. For example, Calbet et al. (2006) examined the associations between Hb_{conc} and aerobic capacity in their review and found that in hemodilutional studies, acute reductions in blood Hb resulted in lower VO_{2max} values and endurance performance without significant changes in blood volume. In turn, increased Hb_{conc} values as a result of blood transfusions caused enhanced VO_{2max}. (Calbet et al. 2006.) Turner et al. (1993) also used blood transfusions to study the role of Hb_{conc} on aerobic performance and found significant increases in VO_{2max} and Hb_{conc} after blood transfusion without concurrent changes in cardiac output, stroke volume or HR.

During the last decade, studies have preferred using Hb_{mass} as the variable describing functional iron levels, rather than Hb_{conc}. Hb_{mass} reflects the total hemoglobin mass in blood and is unaffected by the changes in plasma volume, that often occurs with long-term endurance training (Schmidt & Prommer 2010). It may also be more strongly associated with VO_{2max} since, for example, Hinrichs et al. (2010) who studied elite field hockey players, found Hb_{mass} was correlated with VO_{2max}, while Hb_{conc} and HCT were not. It is generally known that elite athletes tend to have higher Hb_{mass} compared to untrained individuals. In addition, it appears that Hb_{mass} during adolescence might have long-term effects on endurance performance. Wherlin et al. (2016) stated that even after several years of endurance training elite athletes are unable to increase their Hb_{mass} with sea-level training, suggesting that increasing Hb_{mass} is difficult for many athletes. However, during the early years of athletic training, it is still possible to improve Hb count. This was confirmed by Steiner (2019), who studied 16- to 19-year-old elite male athletes for three years and found an 18 % increment in Hb_{mass}. There were also high correlations in Hb_{mass} between ages 16 and 19, suggesting that the Hb_{mass} in adolescents is a strong predictor of future Hb_{mass}. (Steiner 2019.)

Due to the incapability of elite athletes to improve their Hb_{mass} with regular sea-level training, the Hb_{mass} during junior years might have long-term effects on endurance performance and it

may be a predictor of high-level performance in the future. Wherlin & Steiner (2021) studied 16- to 25-year-old elite cross-country skiers and triathletes to examine whether the Hb_{mass} during adolescence was related to national team membership in adulthood. They found that with the 16-year-old juniors who had eventually made it to the national team, the Hb_{mass} was significantly higher than it was for the juniors that terminated their career before the age of 25. Therefore, they concluded that higher body weight related Hb_{mass} in adolescence could be a predictor of transition into a national team level athlete, underlining the importance of monitoring functional iron status and Hb count with junior athletes. (Wherlin & Steiner 2021.)

The associations between storage iron and aerobic performance are also widely studied in non-anemic iron-deficient subjects, especially with female subjects and clear associations have been found between iron depletion and decreased aerobic performance. For example, DellaValle & Haas (2012) found that reduced iron status caused higher blood lactate concentrations during a four-kilometer time trial, lower VO_{2peak} values and an increased duration in time trial completion for female rowers with iron deficiency without anemia. Crouter et al. (2011) also studied iron-depleted, nonanemic females and noted significantly lower VO_2 values at ventilatory threshold after controlling for fat-free mass and vigorous physical activity, but no differences were found in VO_{2max} values. Studies have also examined the role of iron supplementation in order to enhance endurance performance with iron deficient subjects and improvements have been observed. Pasricha et al. (2014) found significant increases in both VO_{2max} and in submaximal exercise performance, defined as lower HR during the exercise, in trained iron deficient female subjects after 6 to 12-weeks of iron supplementation. Similar findings were obtained by Hinton et al. (2000) who detected significantly reduced performance times in 15-kilometer cycle ergometer tests in iron-depleted non-anemic females after a 6-week iron treatment, compared to placebo group.

Despite the results of these studies, iron deficiency does not always lead to changes in physical performance. For example, Telford et al. (1992) failed to find any significant improvements in aerobic and anaerobic power in response to iron treatment. Additionally, Peeling et al. (2007) used intramuscular iron injections in iron-deficient women and even though their serum ferritin levels improved, no changes were observed in aerobic performance, submaximal or maximal VO_2, HR, or blood lactate when compared to placebo group. According to Weaver & Rajaram

(1992), athletic performance is usually affected only after the impairment in erythropoiesis and decrement in hemoglobin content, which is why only a decreased serum ferritin level might not be associated with endurance performance.

Despite the limitations of the presented iron status markers, monitoring of serum ferritin and hemoglobin can provide valuable information regarding the training and recovery status of an athlete. Serum ferritin enables athletes to detect the changes in their iron status before the iron depletion affects erythropoiesis and oxygen transport capacity and thus, endurance performance. Hb_{conc} and Hb_{mass} in turn, are known to be associated with VO_{2max}. Although studies have lately preferred the use of Hb_{mass} as the variable describing functional iron status for endurance athletes, it is still quite complex and an expensive variable to measure. Therefore, for long-term monitoring, Hb_{conc} might be more suitable because of its' increased accessibility. Hb_{conc} also provides important information concerning the severity of iron deficiency (Pfeiffer & Looker 2017) and when detected parallel with HCT, it can be used as an indicator of non-functional fatigue (increased HCT value with concurrent decrement in Hb_{conc}) (Djaoui et al. 2017).

6 RESEARCH QUESTIONS AND HYPOTHESES

The purpose of this study was to examine how aerobic fitness and recovery vary in junior cross-country skiers before and after training season and to detect the relationships between aerobic capacity and the studied recovery markers, HRV and iron status. The study included two testing periods nearly six months apart from each other, where the nocturnal HRV, iron status markers and VO_{2max}, describing the aerobic capacity, were measured and analyzed. Research questions and hypotheses were as follows:

Question 1. How aerobic capacity, HRV and iron status differ before and after the training season in young cross-country skiers?

Hypothesis 1. Higher VO_{2max} values can be observed in the POST measurements.

Increased aerobic training during the training season should lead to enhanced maximal performance and therefore increased VO_{2max}. Although improving the VO_{2max} might not be as easy for already endurance trained athletes (Midgley et al. 2006), the subjects in the present study were still juniors and had not most likely reached the plateau in their VO_{2max}. In addition, studies have shown that regular and even sort-term aerobic training can lead to significant improvements in VO_{2max} even in physically active or already endurance-trained athletes (Tabata et al. 1996; Ní Chéilleachair et al. 2017).

Hypothesis 2. Higher nocturnal HRV values can be observed in the POST measurements.

Six-months regular aerobic training should lead to enhanced aerobic performance which is known to cause adaptations in ANS activity. At rest, such as in nocturnal measurements, HRV should increase, indicating greater parasympathetic activity and lower sympathetic activity, thus contributing to training-induced bradycardia (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013).

Hypothesis 3. Lower Hb_{conc} and s-Ferr levels can be observed in the POST measurements.

In the PRE tests, the subjects had just started their training season, so they should be rested and their iron status parameters should be relatively high. At the end of the measurement period, the training load is increased and intensive training is known to decrease iron stores and increase iron depletion (Ostojic & Ahmetovic 2008) which could lead to decreased Hb_{conc} and s-Ferr levels.

Question 2. What are associations between aerobic capacity, nocturnal HRV and iron status?

Hypothesis 1. Higher nocturnal HRV values are related to higher VO_{2max} values.

Long-term endurance training, that also leads to enhanced $VO_{2max,}$ causes adaptations in ANS activity, and these adaptations cause increments in HRV at rest and during recovery (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013.) Studies have also found clear associations between the changes in aerobic capacity and resting HRV in shorter experiments when using VO_2 at anaerobic threshold to describe the aerobic capacity (Schmitt ym. 2008).

Hypothesis 2. Reductions in Hb and s-Ferr values are related to reduced VO_{2max}.

Iron depletion has been found to be related to reduced VO_{2max} because of the hemoglobin dependent oxygen transportation from respiratory system to working muscles (Crouter ym. 2011; Hinrichs et al. 2010). In hemodilutional studies, reductions in Hb_{conc} have resulted in lower VO_{2max} values, while increased Hb_{conc} values, as a result of blood transfusions, has caused enhanced VO_{2max}. (Calbet et al. 2006.) In the case of serum ferritin, the associations with VO_{2max} have not been as clear, but there is evidence of the negative effect of iron deficiency without anemia on endurance performance (Crouter et al. 2011; DellaValle & Haas 2012).

Hypothesis 3. Higher values in iron status variables are related to higher nocturnal HRV values.

Although the expected changes for HRV and iron status values in the present study are different (increased HRV, decrements in iron status values), it is possible to predict positive correlation between variables. High HRV values describe better recovery status and with higher HRV, one can also expect for enhanced iron status. Negative changes in HRV are caused by stress and disturbed homeostasis which will most likely also occur in decreased iron status values. In addition, both HRV and body iron are related to aerobic capacity (Crouter ym. 2011; Schmitt et al. 2008) and therefore, associations between them can also be expected.

7 METHODS

This study was part of a larger longitudinal study that included two years of testing and monitoring junior cross-country skiers. The present study focused on investigating the changes in aerobic capacity and recovery status in athletes before and after training season, from the beginning of May to the end of October. Incremental exercise test was used to determine VO_{2max} as a measure of aerobic capacity, while the recovery status was estimated by using nocturnal HRV recordings and different iron status biomarkers. In addition, the relationships between these three variables were examined. The study was approved by the Ethical Committee at the University of Jyväskylä and the measurements were performed in accordance with the declaration of Helsinki.

7.1 Subjects

At the beginning of the two years longitudinal study, 30 well-trained junior cross-country skiers were recruited in the study. From these subjects only 12 athletes, 10 females and 2 males, were able to perform all the demanded tests. Because of the small number of male subjects and the large sex differences in iron status metabolism, the two male subjects were excluded, leaving the subject count into 10 females. The subjects were 16-18 years old, first and second grade students in the sports high school of Vuokatti and members of Vuokatti-Ruka Sports Academy. At the beginning of the study, all the subjects were fully informed of all details about the procedures and were told about their freedom to withdraw from the research at any point. Every subject and their parents gave written consent to participate voluntarily.

7.2 Study protocol

The study protocol included two testing periods that were made nearly six months apart from each other. The PRE tests were performed in May after about three weeks into summer training and the POST measurements occurred in late October, right before the competition season. Between the testing periods, the subjects followed their own personal training regimen that was not controlled by the study. However, because all the subjects went to same high school and

were part of the Vuokatti-Ruka Sports Academy, the athletes often trained together and thus, the training remained quite similar. The subjects were supposed to log all their training sessions to their electronic training diaries (elogger.net, Espoo, Finland) and averaged four-week training volumes and intensities were recorded (figure 4).

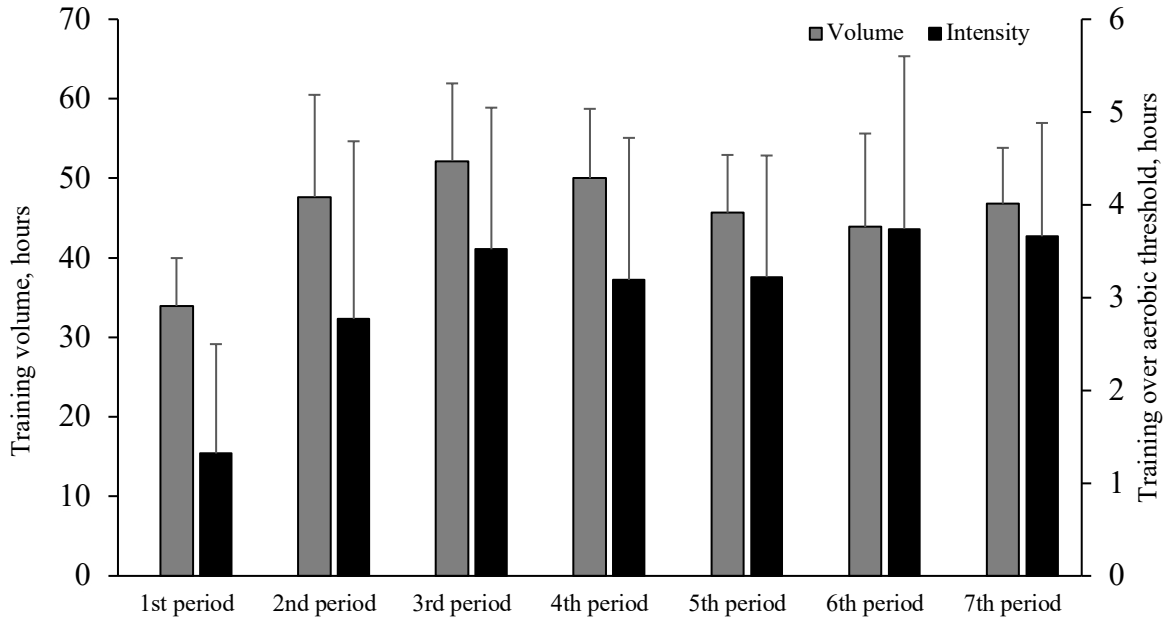


FIGURE 4. Training volume and intensity presented in four-week sections.

The two testing periods lasted from one-to-two weeks and included incremental maximal aerobic fitness test, nocturnal HRV recordings and iron status measurements. For the incremental exercise tests, the subjects were required to commit the tests within two-week period in order to be included in the study. Due to the longitudinal study design, the subjects recorded their nocturnal HRV during the whole two-years research project but for this study, only the weekly average right before the incremental exercise test was used to evaluate HRV. Iron status was estimated from blood samples using several different iron status biomarkers. In the PRE tests, the blood samples were taken one-to-two weeks after the incremental exercise tests and in the POST measurements, the blood samples were taken during the same week as the exercise tests.

7.3 Data collection and analysis

Aerobic capacity. The aerobic capacity was determined with incremental Nordic Walking test in Vuokatti Sport Test Lab. Before the tests, subjects had done their own warm up that was not controlled by the study. The test protocol started with measuring the subjects' height and weight and according to the height, the subjects were given poles. The subjects also committed several static and countermovement jumps and filled stress surveys but their results were not used in this study. After these tests, the subjects were attached to the gas analyzer and their resting HR (Polar V800, Polar Electro Oy, Kempele, Finland) was recorded and blood lactate concentration was collected. The blood lactate concentrations were obtained as blood samples (20 µL) from fingertip and analyzed with Biosen C_line Lactate Analyzer (EKF Diagnostic, Magdeburg, Germany).

The incremental Nordic Walking test included seven three-minute workloads after which the workloads were shortened into two minutes (test sheet presented in appendix 1). During each stage of the incremental test, several variables were recorded including HR, blood lactate and the respiratory gas exchange. HR was continuously monitored and when 15 seconds of each load remained, HR values were recorded. Blood lactate was analyzed from the blood samples that were collected during the last 30 seconds of each stage, while the workload remained the same. After reaching the subjects evaluated anaerobic threshold (1.04 RER value), the blood samples were no longer collected. The gas exchange data was recorded breath-by-breath using the Jaeger Oxygen Pro (Viasys Healthcare GmbH, Hoechberg, Germany) and the mean values of different variables were calculated as 30-second averages.

The incremental exercise test ended to exhaustion when the subjects could no longer keep up with the treadmill or decided to stop the test. After the treadmill stopped, subjects did a 10-minute cool-down on the treadmill that started immediately after the subjects were able to start walking. During the cool-down, blood lactate concentrations were collected 1, 4, and 10 minutes after the test had ended.

Heart rate variability. The nocturnal heart rate variability was measured with a contact-free sleep tracking Emfit QS-device (Emfit QS, Kuopio, Finland). The device measures sleep and HR variables with a pressure sensor that is placed beneath the mattress under the subject's chest area. It starts recordings automatically when the subject goes to sleep. The collected data was transferred and stored online via internet. Emfit QS reported the magnitude of HRV with a time-domain variable RMSSD with the sampling rate of 100 Hz.

The subjects were given the Emfit QS-device at the beginning of the two-years longitudinal study already in February 2019, so they were able to record their nocturnal HRV during the whole six-months research period. However, in this study, the data was only analyzed from the week before the exercise tests. The data was analyzed as a weekly average so that at least in three out of seven nights the recorded data had been successful.

Time domain variable RMSSD was used to determine HRV. The average of morning and evening RMSSD values, that describe the average of all three-minute window RMSSD values measured during first 90 minutes after falling asleep and last 90 minutes before waking up, were used in the analysis to describe nocturnal HRV. The validity of the Emfit QS in measuring HR and HRV was proven by Vesterinen et al. (2020), who found large correlations in mean values of the sleep period in HR ($r=0.90$, $P<0.001$) and Ln (natural logarithm) RMSSD ($r=0.89$, $P<0.001$), and only small bias in the mean HR (mean –0.8 bpm, SD 2.3 bpm, $P=0.15$) and Ln RMSSD (mean –0.05 ms, SD 0.25 ms, $P=0.33$) between the Emfit QS device and ECG-based reference device, Firstbeat BG2 (Vesterinen et al. 2020). The reliability of the Emfit QS device in measuring HR and HRV has not been proven yet.

Iron status measurements. Iron status was evaluated by using hemoglobin, hematocrit and serum ferritin obtained from blood samples from the antecubital vein. The PRE and POST samples for each subject were obtained at the same time of day between 7 a.m. and 9 a.m. In the PRE tests, the blood samples were collected one-to-two weeks after the VO_{2max} test while in the POST tests, the samples were collected during the same week as the incremental exercise test.

For the analysis of hemoglobin and hematocrit the collected blood was drawn into EDTA tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) and immediately further analyzed with Sysmex XP300 analyzer (SysmexCo., Kobe, Japan). For serum ferritin, the blood was drawn into Vacuette gel serum tubes (Greiner-Bio-One GmbH, Kremsmünster, Austria) and centrifuged for 10 minutes with 3600 rpm to collect serum, which was then frozen to -20 ºC. The samples were analyzed with Siemens Immulite 2000 XPI analyzer (Siemens Healthcare Lianberis, United Kingdom) where the serum ferritin was determined by using immunometric chemiluminescence method. The sensitivity of the assay for ferritin was 0.4µ/l and the precision (CV%) for the assay was 4.6%.

7.4 Statistical analysis

All the statistical analysis was performed with SPSS for Windows software (IBM SPSS Statistics 26 (SPSS, Inc., Chicago, Illinois, USA)). Shapiro-Wilk's test was used to test whether the data was normally distributed and due to small sample sizes, non-parametric methods were used for the statistical analyzes. Wilcoxon's Signed Rank test for two-related samples was performed to analyze the changes between the PRE and POST tests in aerobic capacity, HRV and iron status values. To analyze the relationships between variables, the bivariate correlation test (Spearman's correlation) was used which is also suitable for small sample sizes. Before the bivariate analysis, the repeated measures data was first transformed into difference variables and then the relative changes were computed (percentage change from the PRE test value) to detect the associations between the concurrent changes between different variables. The results are presented as means ± standard deviations.

8 RESULTS

8.1 The changes in aerobic capacity, nocturnal HRV and iron status

The six-month training-season caused no significant changes in VO_{2max}, the measure of aerobic capacity. There were also no significant changes in the recovery markers nocturnal HR and HRV or in iron status variables. The averaged values of the measured variables in the PRE and POST measurements are presented in table 3 and figures 5 and 6. To demonstrate the magnitude of the changes, the average relative change ($p > 0.05$) within the variables were also calculated and are shown in table 4 and figure 7.

TABLE 3. Mean (±SD) HR, RMSSD, VO_{2max} and iron status indices in the PRE and POST measurements.

	PRE	POST
HR (bpm)	53 ± 3	55 ± 7
RMSSD (ms)	66 ± 10	68 ± 13
VO_{2max} (ml/kg/min)	55 ± 2	56 ± 3
Hb_{conc} (g/l)	138 ± 8	141 ± 6
HCT (%)	43 ± 2	43 ± 2
s-Ferr (µg/l)	47 ± 18	41 ± 23

HR, heart rate; RMSSD, the square root of the mean squared differences of successive R-R intervals; VO_{2max}, maximal oxygen uptake; Hb_{conc}, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin.

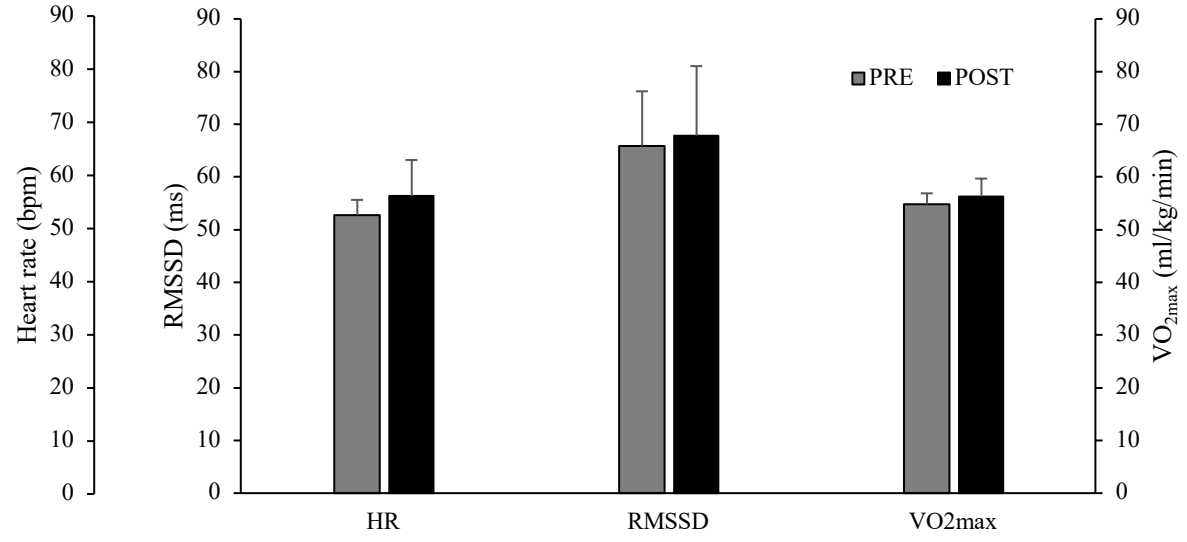


FIGURE 5. Mean (±SD) nocturnal HR, RMSSD and VO_{2max} in the PRE and POST measurements. HR, heart rate; RMSSD, the square root of the mean squared differences of successive R-R intervals; VO_{2max}, maximal oxygen uptake.

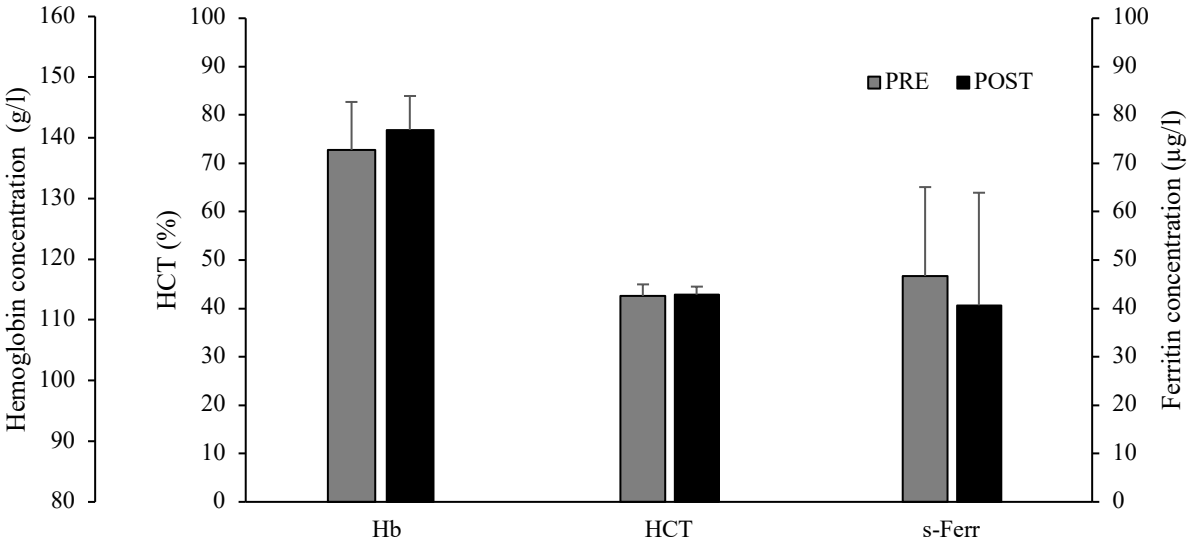


FIGURE 6. Mean (±SD) Hb_{conc}, HCT and s-Ferrin in the PRE and POST measurements. Hb, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin.

TABLE 4. Mean (±SD) changes (%) in nocturnal HR and RMSSD, VO_{2max} and iron status indices between the PRE and POST measurements.

	HR	HRV	VO_{2max}	Hb_{conc}	HCT	s-Ferr
Percentage of change	6.8 ± 10.3	4.2 ± 23.0	2.7 ± 4.5	2.5 ± 3.3	0.8 ± 3.8	6.1 ± 41.9

HR, heart rate; RMSSD, the square root of the mean squared differences of successive R-R intervals; VO_{2max}, maximal oxygen uptake; Hb_{conc}, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin.

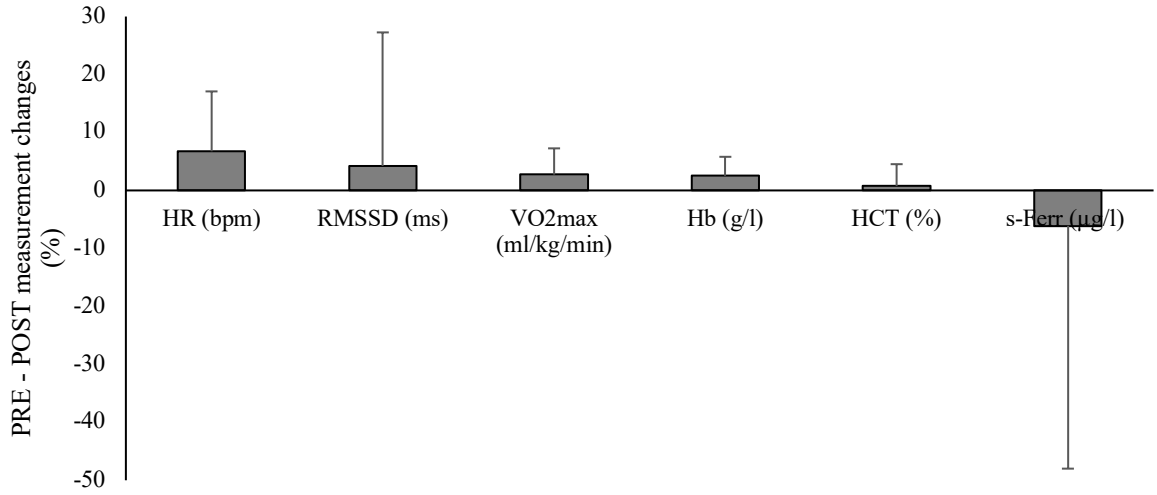


FIGURE 7. Mean (±SD) changes (%) in nocturnal HR and RMSSD, VO_{2max} and iron status indices between the PRE and POST measurements. HR, heart rate; RMSSD, the square root of the mean squared differences of successive R-R intervals; VO2max, maximal oxygen uptake; Hb, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin.

8.2 Relationships between aerobic capacity, nocturnal HRV and iron status

There were significant correlations between HRV and iron status, of which the most significant correlation was observed between HRV and Hb_{conc} ($r = 0.796$, $p = 0.006$). There were also positive correlations between HRV and HCT ($r = 0.717$ $p = 0.02$) and between Hb_{conc} and HCT ($r=0.685$, $p=0.029$). Storage iron variable s-Ferr and VO_{2max} did not show any associations with

the studied markers. The correlation coefficients for relative changes are presented in table 5. The scatterplots of the relationships between the relative changes in HRV and Hb_{conc} and between HRV and HCT are shown in figure 8.

TABLE 5. Spearman's bivariate correlations between the studied variables.

		HR	HRV	Hb_{conc}	HCT	s-Ferr
HRV	r	-0.310				
Hb_{conc}	r	-0.588	0.796**			
HCT	r	-0.333	0.717*	0.685*		
s-Ferr	r	-0.527	0.090	0.442	0.018	
VO_{2max}	r	-0.313	0.086	0.350	0.522	-0.055

* $p < 0.05$, ** $p < 0.01$, statistically significant correlation between difference variables. HR, heart rate; HRV, heart rate variability; Hb_{conc}, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin; VO_{2max}, maximal oxygen uptake.

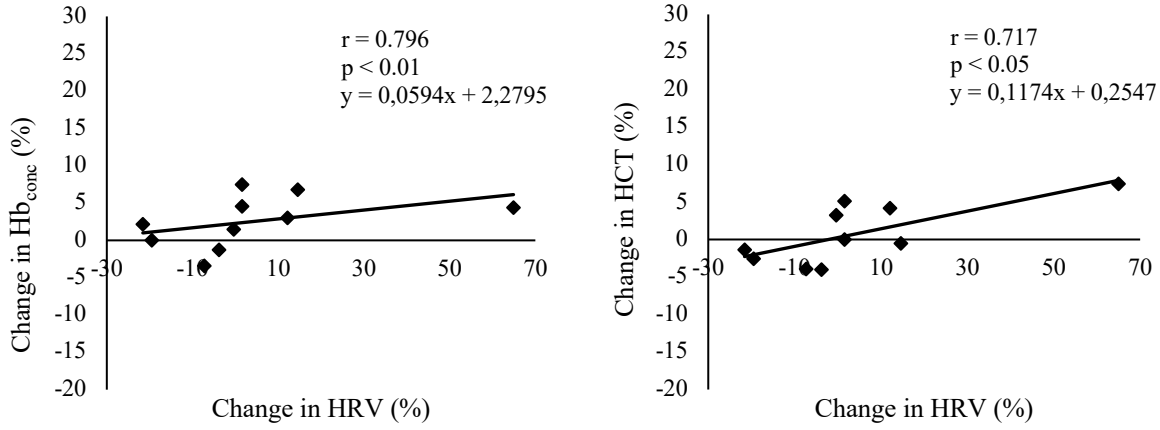


FIGURE 8. Scatterplots of the relationships between relative changes in HRV and Hb_{conc} (left figure) and between the relative changes in HRV and HCT (right figure). HRV, heart rate variability; Hb_{conc}, hemoglobin concentration; HCT, hematocrit.

There were only two subjects whose Hb_{conc} decreased during the measurement period. For these two athletes, all the other recovery markers were also impaired in the POST measurements and

VO_{2max} either decreased or remained unchanged. For the other subjects, changes in the recovery markers were more diverse. Table 6 summarizes all the relative within-subject changes in different variables (% change from the PRE test values) that occurred during the measurement period. A positive change represents higher values in the POST measurements, while negative value implies lower POST values.

TABLE 6. Within-subject changes (%) in the studied variables between the PRE and POST measurements.

Subject	VO_{2max}	HR	HRV	Hb_{conc}	HCT	s-Ferr
1	-5.5	10.5	-7.3	-3.4	-3.9	-18.7
2	0.0	6.6	-3.8	-1.3	-4.0	-54.1
3	0.0	0.9	14.6	6.8	-0.5	78.0
4	0.0	8.2	-19.5	0.0	-2.6	27.4
5	1.8	33.5	64.9	4.4	7.4	-54.7
6	1.9	-2.0	12.1	3.0	4.2	-2.7
7	3.8	-4.9	1.6	7.5	5.1	41.7
8	5.5	12.1	-21.6	2.2	-1.4	-33.6
9	8.9	0.7	-0.4	1.5	3.2	-44.0
10	10.9	2.0	1.6	4.6	0.0	-0.3

VO_{2max}, maximal oxygen uptake; HR, heart rate; HRV, heart rate variability; Hb_{conc}, hemoglobin concentration; HCT, hematocrit; s-Ferr, serum ferritin.

9 DISCUSSION

The aims of this study were to examine how aerobic fitness and recovery vary in junior female cross-country skiers before and after a training season and to detect relationships between aerobic capacity and the studied recovery markers, HRV and iron status. The main finding of the present study was that there is an association between the changes in HRV and functional iron Hb_{conc}. In addition, the results suggest that impaired Hb_{conc} might affect maximal aerobic performance negatively by hindering the training-induced performance gains.

9.1 The changes in aerobic capacity, nocturnal HRV and iron status

In the present study, the first research question considered the changes in aerobic capacity and recovery markers (HRV and iron status) during a six-month training season in junior cross-country skiers. Contrary to the expectations, there were no significant changes in any of the measured variables.

Aerobic capacity. VO_{2max}, the variable representing aerobic capacity, increased approximately by 2.7 % during the six-month training-period. The corresponding 1.5 ml/kg/min raise in VO_{2max} is actually quite a large change in such a short time with already endurance trained athletes but, in this study, it was not enough to be considered significant.

VO_{2max} was expected to increase to the POST measurements since increased aerobic training is known to lead to enhanced maximal performance even with trained athletes (Tabata et al. 1996; Ní Chéilleachair et al. 2017). Moreover, junior athletes have most likely not yet reached their maximal potential in their aerobic capacity (the plateau in VO_{2max}) and therefore, should be able to enhance their VO_{2max} during six-months of intensive aerobic training. The subjects averaged training data shows how both the training volume and intensity increased during the training season. Before and during the PRE tests training volume and the amount of intensive training were still very low, since the athletes were still slowly coming back to their normal training regimen after approximately one month of reduced training due to an off-season. After the first four-week period, the training volume was highly increased, and it was more that 32 % higher

for each of the following four-week periods. The amount of intensive training was also at its lowest right before the first test-week and increased linearly until it was nearly two-and-a-half-times higher during the last two four-week periods.

One of the reasons, that could explain the insignificance of the changes in VO_{2max} despite the increased training load, could be the small number of subjects. There were only 10 subjects in the study, of which six were able to improve their VO_{2max}. Even though only one subject displayed a decrease in VO_{2max}, the averaged change in the whole study group remained relatively low. Another explanatory mechanism could be the large variation in the VO_{2max} response to similar training program, that the subjects committed as they part of the same sports academy. Studies have shown, that the same training program can induce variety of responses in performance and physical adaptations in different people (Granero-Gallegos et al. 2020). It is possible that while the highly increased training load was appropriate for some of the subjects, for the others the training was too hard or not hard enough, which led to hindered performance gains.

Nocturnal heart rate variability and heart rate. Contrary to our hypothesis, there were no remarkable changes in nocturnal measurements. The nocturnal HRV increased approximately by 4.2 % but was likely influenced by the small number of subjects and large variation in HRV changes (SD = ± 23 %), hence, the difference remained insignificant. However, since there were only minor changes in VO_{2max}, it is understandable that the HRV also remained close to the PRE test values. Improvements in VO_{2max} and endurance performance are known to cause long-term adaptations in the ANS activity, and these adaptations should be seen as enhancements in the HR parameters at rest and during recovery, such as during nocturnal measurements (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013). Since there were no recognizable improvements in aerobic capacity, changes in nocturnal HRV cannot be expected.

Slightly conflicting changes were observed with nocturnal measurements, since even though HRV was slightly improved, there was an increase in HR (6.8 %). Similar to HRV, nocturnal HR is known to adapt to long-term endurance training but instead of increasing, it should

decrease (Saltin et al. 2000, 230-232). Higher resting HR is a sign of stress and disturbed homeostasis and it is known that excessive endurance training can lead to suppressed PNS activity and, therefore, increase HR and decrease HRV even at rest (Baumert et al. 2006; Hynynen et al. 2007; Pichot et al. 2000; Plews et al. 2014).

The reason why nocturnal HR increased to the POST measurements, while HRV improved, may be due to the notably higher training load and intensity right before the second testing-period. Previous studies have revealed similar findings before and, for example, Martinmäki et al. (2012) noted that nocturnal HR increased after elevated exercise intensity, while HRV decreased only when exercise duration was long enough. Therefore, it can be concluded that the increased exercise intensity before the POST measurements might have had an effect on nocturnal HR but the training load was not high enough to affect HRV.

Iron status values. There were also no significant changes ($p > 0.05$) in any of the iron status variables. Hb_{conc} increased by 2.5 % and s-Ferr decreased by 6.1 %, while HCT was almost unaffected (0.8 %). A stable HCT implies, that the portions of red blood cells and plasma in circulation did not change during the six-months training period. This in turn confirms, that the changes in s-Ferr and Hb_{conc} were accurate and not affected by the changes in HCT.

The s-Ferr and Hb_{conc} were expected to decrease in the POST measurements as a consequence of an increased training load. During the PRE tests, the subjects had just started their training season, implying that the subjects should have been rested and their iron status parameters relatively high. With a highly increased amount of training, especially intensive training, iron depletion is usually increased and iron stores decreased, leading to decreased s-Ferr and Hb_{conc}. (Ostojic & Ahmetovic 2008). Although the average s-Ferr was slightly lower in the POST measurements, the detection of individual cases reveals, that the variation in the relative changes was really high. Since there were no distinguishable trend in the changes of s-Ferr during the training season, it seems, that storage iron levels are not necessarily affected by the changes in training load.

The changes in Hb_{conc} also remained insignificant and even the small average change was contrary to the one expected, since seven of the ten subjects had increased Hb_{conc} values. A possible explanation for the small increase in Hb_{conc}, while s-Ferr levels were slightly impaired, is that the training load had been high enough to start the iron depletion process from the iron stores but it had not lasted for so long that it would have also affected functional iron levels and Hb_{conc}. Iron depletion occurs gradually starting from the depletion of storage iron, after which the amount of transport iron and finally the content of functional iron is depleted (Garcia-Casal et al. 2018). Studies have found that s-Ferr stores can be depleted before critical declines in Hb_{conc} (Lee et al. 2017) and since in this study, the s-Ferr levels for all the subjects were still above its cut-off values (appendix 2), the decrement did not affect functional iron content.

One factor that could have also affected iron status values is the possible role of dietary iron. Oral iron supplementation can promote iron absorption and, therefore, help to maintain or even increase Hb_{conc} (Lee et al. 2017; Magazanik et al. 1991; Stoffel et al. 2020). The study did not control the subjects' supplementation and it is probable that the subjects had consumed iron during their hard training weeks. The subjects had been instructed not to digest any iron at least for a week before the iron status measurements, but it is possible that earlier supplementation still had an increasing effect on the athletes' Hb_{conc}.

9.2 Relationships between aerobic capacity, nocturnal HRV and iron status

The most notable association between the different variables was the positive correlation between the relative changes in Hb_{conc} and HRV. In addition, there were positive correlations between HCT and HRV and between HCT and Hb_{conc}. The latter was highly expected since HCT describes the volume of blood cells in circulation, mainly red blood cells where most of the hemoglobin is located. A positive correlation between HRV and iron values was also expected since those two markers are supposed to reflect changes in recovery status similarly by decreasing in response to physical stress (Achten & Jeukendrup 2003; Fatisson et al. 2016; Ostojic & Ahmetovic 2008).

Another intriguing finding in the study was that the two subjects whose Hb_{conc} decreased in the POST measurements also displayed either a decreased or unchanged VO_{2max}, despite the six-months of intensive aerobic training. The two subjects with decreased Hb_{conc} values had also impaired results in all the other recovery markers. Although no correlations were found between VO_{2max} and iron status variables, the results suggest that an impaired functional iron status might hinder the improvements in aerobic capacity.

Relationships between VO_{2max} and heart rate variability. Despite the findings in earlier literature, there was no correlation between VO_{2max} and HRV in the present study. Long-term endurance training is known to cause adaptations in ANS activity which should lead to increased PNS activity and HRV at rest (Achten & Jeukendrup 2003; Carter et al. 2003; Stanley et al. 2013). Even though HRV was slightly higher in the POST measurements, there was large variation in the HRV changes and half of the subjects displayed impaired HRV. The large variance in the subjects HRV changes inhibited the associations with the changes in VO_{2max}. The decrements in HRV values are most likely caused by the relatively high training loads right before the second measurement-period. To avoid these kinds of training-induced changes in the ANS variables, the subjects training should have been more controlled at least a week prior to the testing period to allow for similar conditions during PRE and POST measurements.

Relationships between VO_{2max} and iron status. There were no significant associations between VO_{2max} and any of the iron status variables. Although some studies have found positive effects of higher Hb_{conc} on aerobic capacity, they have occurred mainly after blood transfusions in subjects suffering from iron deficiency (Calbet et al. 2006.; Turner et al. 1993). Therefore, a linear correlation between enhanced iron status and aerobic capacity cannot be expected, since increments in iron status in already heathy subjects will most likely not affect maximal aerobic performance.

Instead, decrements in iron status might affect aerobic capacity because it is a sign of a non-homeostatic state of the body that could also affect aerobic performance. Similar types of aerobic performance diminishing effects of iron depletion have been confirmed by several studies detecting female endurance athletes (Crouter et al. 2011; DellaValle & Haas 2012).

Unfortunately for this study, the small number of subjects made it impossible to make any sub-group analyzes for the subjects whose s-Ferr or Hb_{conc} was diminished. Some notifications can still be made from the difference variables by detecting individual cases. The results show that there were only two subjects whose Hb_{conc} values decreased and these subjects had impaired results also in all the other recovery markers. In turn, their VO_{2max} was either impaired or unchanged, while most of the subjects were able to increase their VO_{2max} (2.7 % increment in whole study group). These findings suggest that even though no correlations were found between VO_{2max} and iron status variables, some associations between the functional iron levels and aerobic capacity might exist and even small decrements in Hb_{conc} could be regarded as a warning sign of insufficient recovery that can affect aerobic capacity.

S-Ferr, the measure of storage iron, did not appear to associate with changes in VO_{2max}, since there were quite large decrements also in subjects who were able to improve their VO_{2max}. This can be explained with the fact that the s-Ferr levels stayed above its cut-off values for all the subjects and, therefore, the decrement did not affect functional iron levels that could have led to impairments in aerobic capacity.

Relationships between HRV and iron status. A remarkable positive correlation was found between HRV and Hb_{conc}, as well as between HRV and HCT. These associations were expected since both ANS functions and iron status are expected to reflect changes in recovery status and homeostasis similarly by decreasing during stress. Specifically, the strong correlation between HRV and Hb_{conc} emphasizes that changes in the ANS functions can reflect changes in functional iron levels. Because HRV is non-invasive and easier to monitor in daily basis than iron status, the changes in HRV values might serve as a new monitoring tool for functional iron levels of which impairment can lead to decreased aerobic performance. These associations have not been studied on healthy athletes before, meaning that the finding is relatively new information in the field of exercise science.

S-Ferr levels did not show associations with the changes in HRV. Similar to the conclusion made about the associations between s-Ferr and VO_{2max}, it is possible that as long as the storage

iron levels remain above cut-off values, the changes in s-Ferr will not occur concurrently with the changes in the ANS activity and HRV.

9.3 Limitations

There were several limitations in the study that might have affected the results. The first limitation is the small number of subjects, which causes the statistical analysis to underestimate the significance of both seasonal changes and relationships between different variables. Especially seasonal changes, where no significant differences were found, was incurred by the small number of subjects.

Since there are gender-related differences in iron metabolism and absolute VO_{2max} values, it was good to have a homogenous group that included only female subjects. However, when examining relationships between changes in different variables, it would have been interesting to have another subject group from males to detect if there would have been similar associations between iron status and HRV. Therefore, further research is needed to verify, if similar association could be found in a healthy athletic male population.

As already discussed, the subjects' preparation for the measurements should have been more strictly planned. The preparation week before the PRE and POST measurements should have been controlled to avoid the possible effects of exercise-induced stress on the ANS functions which would have made the values more comparable. Also, the ingestion of dietary iron should have been controlled since it is impossible tell for sure if it had an effect on the subjects' iron statuses.

Some of the limiting factors were associated with the data collection process. There were many problems in connecting the Emfit QS-devices to internet, which led to missing data. The solution was to exclude the cases who had less than four nights of successful HRV recordings within one week before the VO_{2max} test, which limited the number of subjects. In addition, since all the nights during the measurement week were not included, the averaged HRV data might have been slightly affected.

The use of Hb_{conc} as a marker of functional iron can also be questioned because of the endurance training-caused expansions in plasma volume. Especially, when studying the associations between Hb_{conc} and VO_{2max}, one might not be able to find increments in absolute Hb protein count because of the concurrent increment in plasma volume (Hinrichs et al. 2010). Obtaining Hb_{mass} could be a better method to evaluate functional iron levels, especially in endurance athletes, but the procedure of measuring the Hb_{mass} is quite complex. Therefore, it is not often monitored in athletes, especially with juniors. Hb concentration in turn is easy to obtain, comparable to the earlier measurements and a more practical variable that athletes are known to follow in their normal training regimen.

9.4 Conclusions and practical applications

The results of this study verify the assumption that there are associations between the ANS activity and iron metabolism in female subjects. Especially the changes in functional iron Hb_{conc} seems to be associated with the changes in HRV. Since HRV is easier to monitor on a daily basis than iron status variables, the monitoring of HRV could be used as a tool to avoid decreasing changes in functional iron, of which impairment can lead to decreased aerobic performance.

Another intriguing finding in the study was that when the Hb_{conc} decreased, VO_{2max} values were either impaired or remained unchanged. The inability to improve VO_{2max} even after six-months of intensive aerobic training with concurrent impairment in functional iron status leads to assumption, that an impaired functional iron status might hinder the improvements in aerobic capacity. Therefore, endurance athletes should react even on small decrements in Hb_{conc}, ensure a sufficient amount of recovery and consider consuming iron supplements to avoid the possible performance diminishing effects of impaired functional iron status. The decrements in s-Ferr do not, however, seem to be harmful as long as the levels stay above cut-off values.

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APPENDIX 1. Test sheet in incremental aerobic fitness test.

INCREMENTAL NORDIC WALKING TEST

DATE:

WEIHG: SSN:

NAME: POLE: HEIGHT:

Time	Speed	Incline	Work ml	HR	La	VE (l/min)	% O2	VO2 (l/min)	ml/kg	RER	Efficiency
Rest											
0-3	5,0	3,5	20								
3-6	5,5	4,9	26								
6-9	6,0	6,1	32								
9-12	6,0	8,0	38								
12-15	6,0	9,9	44								
15-18	6,0	11,7	50								
18-21	6,0	13,6	56								
21-23 (*2')	6,5	14,0	62								
23-25 (*2')	7,0	14,3	68								
25-27 (*2')	7,5	14,6	74								
Time:											

	AerT	AnaT	Max V_{O2}		Lactate clearance	
l/min					1 min	
ml/kg					4 min	
Work (work/time)					7 min	
HR_{max}					10 min	
La					La-clearance (mmol/l/min)	

APPENDIX 2. PRE and POST measurements values of nocturnal HR, HRV, VO_{2max} and iron status indices Hb, HCT and s-Ferr.

Subject	HR (bpm)		HRV (RMSSD, ms)		VO_{2max} (ml/kg/min)	
	PRE	POST	PRE	POST	PRE	POST
1	50.1	55.4	78.5	72.7	55	52
2	58.9	62.8	78.0	75.1	56	56
3	52.8	53.3	56.5	64.7	59	59
4	55.0	59.5	60.8	49.0	52	52
5	52.2	69.7	50.3	83.0	55	56
6	49.9	48.9	72.0	80.6	52	53
7	52.6	50.0	70.5	71.6	53	55
8	55.3	62.0	54.0	42.4	55	58
9	51.0	51.3	75.5	75.2	56	61
10	50.0	51.0	63.3	64.3	55	61

HR, heart rate; HRV, heart rate variability; VO_{2max}, maximal oxygen uptake.

Subject	Hb (g/l)		HCT (%)		s-Ferr (µg/l)	
	PRE	POST	PRE	POST	PRE	POST
1	146	141	45.7	43.9	28.3	23.0
2	156	154	47.8	45.9	51.0	23.4
3	133	142	41.0	40.8	15.9	28.3
4	140	140	42.8	41.7	78.5	100.0
5	137	143	41.9	45.0	52.8	23.9
6	134	138	40.7	42.4	43.9	42.7
7	134	144	41.1	43.2	38.4	54.4
8	139	142	43.0	42.4	65.8	43.7
9	131	133	40.8	42.1	57.3	32.1
10	130	136	41.0	41.0	35.2	35.1

Hb, hemoglobin; HCT, hematocrit; s-Ferr, serum ferritin.